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(54) Title: COMPOSITIONS AND METHODS FOR MODIFYING AN IMMUNE RESPONSE AGAINST TROPOMYOSIN

(57) Abstract: Disclosed are compositions and methods for modifying an immune response against tropomyosin and related antigens. In one embodiment, the invention provides vaccines including modified tropomyosin that reduce or eliminate an unwanted immune response against tropomysin. Also provided are modified tropomysin molecules with modified antibody binding sites (epitopes) that significantly reduce or eliminate potential to engage the immune system. The invention also provides transgenic animals, particularly transgenic shrimp, that include at least one of the modified tropomysin molecules described herein. The invention has a wide spectrum of uses including reducing harmful immune responses to crustacea, anthropods, and other animals.

COMPOSITIONS AND METHODS FOR MODIFYING AN IMMUNE RESPONSE AGAINST TROPOMYOSOSIN

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to compositions and methods for modifying an immune response against tropomyosin and related antigens. In one embodiment, the invention provides vaccines including modified tropomyosin that reduce or eliminate an unwanted immune response against tropomyosin. Also provided are modified tropomyosin molecules with modified antibody binding sites (epitopes) that significantly reduce or eliminate potential to engage the immune system. The invention also provides transgenic animals, particularly crustacea, that includ at least one of the modified tropomyosin molecules described herein. The invention has wide applicability including reducing harmful immune responses to crustacea, arthropods, and other animals.

2. Background

Allergic disease is a common health problem affecting humans and companion animals (mainly dogs and cats) alike. Allergies exist to foods, molds, grasses, trees, insects, pets, fleas and other substances present in the environment. Some allergic reactions, especially to foods and insects can be so severe so as to be life threatening.

There is almost universal recognition that most allergies are manifested by the release of histamines and other mediators of inflammation by mast cells and basophils which are triggered into action when IgE antibodies bound to their receptors on the mast cell surface are cross-linked by allergan. Other than avoidance, and drugs, for example, antihistamines, decongestants, and steroids, that only treat symptoms and can have unfortunate side effects and often provide only, temporary relief, the only currently medically accepted treatment for allergies is immunotherapy. Immunotherapy involves the repeated injection of allergan extracts over a period of years to desensitize a patient to the allergan. Unfortunately, traditional immunotherapy is time consuming, usually involving

years of treatment, and often fails to achieve its goal of desensitizing the patient to the allergan.

Among allergans of animal origin, shellfish (crustaceans and mollusks) are a frequent cause of allergic reactions to foods. Most shellfish species that elicit allergic food reactions belong to the class crustacea and include shrimp, crab, crawfish and lobster; the shrimp genera *Penaeus* and *Metapenaeus* have been two of the most frequently studied. The only major allergan reported in shrimp is the muscle protein tropomyosin. At least 80% shrimp-allergic subjects react to tropomyosin and it binds approximately 85% of the shrimp specific IgE from shrimp-allergic subjects; all other shrimp allergans bind IgE from less than 25% of the shrimp-allergic subjects. Recent studies have demonstrated that tropomyosin is an important allergan in other crustaceans such as lobster *Panulirus stimpsoni* and *Homarus americanus* (Pan s 1, Hom a 1) , crab *Charyabdis feriatus* (Cha f 1), mollusk such as squid *Todareus pacificus* (Tod p 1), snail *Turbo cornutus* (Tur c 1) and oyster *Crassostrea gigas* (Cra g 1) and in other invertebrates such as house dust mite *Dermatophagoides farinae* (Der f 10) and *D. pteronyssinus* (Der p 10) , and cockroach *Periplaneta americana* (Per a 7). Furthermore, concomitant clinical and *in vitro* hypersensitivity to crustaceans, insects, arachnids, mollusks and even nematodes have implied tropomyosins as the cause of clinical crosssensitivity among invertebrates.

It has been reported that tropomyosin belongs to a family of proteins present in all eukaryotic cells, where it is associated with the thin filament in muscle, and microfilaments in many non-muscle cells. Together with actin and myosin, tropomyosin plays a role in the contractile activities of these cells, as well as in the regulation of cell morphology and motility. Tropomyosin is thought to be present in phylogenetically unrelated vertebrate and invertebrate species, with several tropomyosin isoforms being found in muscle (skeletal, cardiac and smooth), and non-muscle cells such as those in brain, fibroblasts and platelets. Even though the degree of sequence identity and functional similarity is reported to be very high among tropomyosins, vertebrate tropomyosins are generally considered to be non-allergenic.

Tropomyosins have attracted substantial research interest.

For example, there is understanding that the proteins are coiled-coil dimers made up of two parallel α-helical tropomyosin molecules that are wound around each other. The tropomyosin monomer contains a heptad repeat, (abcdefg), in which generally large hydrophobic non-polar residues occur at positions a and d, while positions b, c, e, f and g are usually occupiedby polar or ionic amino acids. The interaction between two alpha-helices in a coiled-coil involve these hydrophobic residues in position a and d. Also charge-charge interactions between acidic residues found in position e and basic residues in position g help also stabilize the coiled-coil. Outer positions b, c, f, must be free to interact with proteins such as actin and troponin. Interestingly, the tandem repeats always occupy position d, e, f, g, and a in the heptameric repeats, which represents the area of interaction between the two α-helixes of tropomyosin. Although tropomyosin is a very flexible molecule that may spontaneously unfold and expose those internal sequences, the repeats are thought to be mostly non-exposed sequences in the native molecule.

Tests such as skin prick test (SPT) have been used to gauge risk of allergy to tropomyosin and other molecules.

There remains a need for a safe and efficacious therapy for allergies, especially where traditional immunotherapy is ill advised due torisk of anaphylaxis to the patient or lack of efficacy. There is also a need for alternatives to therapies, for creating foods, materials or substances which contain modified allergans that do not elicit a harmful allergic response.

SUMMARY OF THE INVENTION

The invention generally relates to compositions and methods for modifying an immune response against tropomyosin. As discussed below, the invention has many important uses including reducing or eliminating harmful immune responses against the tropomyosin of animals such as crustacea and arthropods. Also provided are transgenic animals and especially transgenic crustacea that include at least one recombinant tropomyosin specifically modified to reduce or eliminate the unwanted immune responses.

In particular, we have discovered tropomyosin sites (epitopes) that engage the vertebrate immune system and elicit harmful immune responses. More specifically, we have identified the Pen a 1(tropomyosin) epitopes that elicit IgE-

mediated immune responses. As related below, we have found about 15 - 20 IgE binding regions in the tropomyosin molecule. We have found one epitope for region 1, one epitope for region 2, two epitopes for region 3, one epitope for region 4 and three epitopes for region 5.

It is thus an object of the invention to provide tropomyosin molecules in which at least one of the IgE binding regions has been modified to reduce or eliminate IgE antibody binding.

Accordingly, and in one aspect, the invention provides vaccines that include at least one modified tropomyosin that reduces or eliminates an unwanted immune response against tropomyosin. Such a modified tropomyosin can, in one embodiment, include at least one amino acid substitution in at least one of the foregoing regions one to five of tropomyosin. Other vaccines according to the invention will include at least a fragment of tropomyosin and may also include at least one of the modified tropomyosins described herein.

In a particular embodiment, the vaccine will include at least one modified amino acid that reduces binding, preferably specific, between crustacea tropomyosin and an IgE antibody of interest by at least about 45% as determined by a standard IgE antibody test. Preferably, suitably modified amino acid sequences will include at least one epitope with at least one amino acid substitution that reduces the binding according to the test. Examples of such preferred amino acid substitutions include those which remove at least one of a non-polar aliphatic, polar uncharged, aromatic, positively charged or negatively charged group from the amino acid.

As discussed, another preferred vaccine includes crustacea tropomyosin or an IgE antibody eliciting fragment thereof. In this invention embodiment, exposure to the tropomyosin is thought to condition the immune system against significantly engaging the molecule as part of a harmful response.

Also provided are modified tropomyosin molecules such as peptides with modified antibody binding sites (epitopes) that significantly reduce or eliminate potential to engage the immune system. The invention also provides transgenic animals, particularly crustacea, that include at least one of the modified tropomyosin molecules described herein. The invention has wide applicability

including reducing harmful immune responses to crustacea, arthropods, and other animals.

Tropomyosin is an essential muscle protein that is present in all animal species and is highly conserved. Thus, invertebrate tropomyosins have amino acid sequence homology with vertebrate tropomyosins such as those present in non-allergenic foods such as beef, pork and chicken. We have used the homologous region of non-allergenic tropomyosin as a template to alter the allergenic epitopes of shrimp tropomyosin to render them inactive. The aim was to make minimal changes in the allergenic epitopes that would induce maximal reduction of tropomyosin-specific IgE binding.

In one embodiment, the invention provides a series of 46 overlapping peptides that span the entire 284 amino acid residue of Pen a 1 tropomyosin protein. Each of these peptides were used to identify the IgE binding epitopes of Pen a 1. As used herein, IgE binding epitopes are defined as any sequence of Pen a 1 that binds Pen a 1-specific IgE of shrimp allergic subjects.

In another embodiment, the invention provides for peptides that have reduced or totally lack binding ability to Pen a 1-specific IgE. Peptides were generated that had one or more amino acid substitutions based on, but not limited to, sequence comparisons with non-allergenic tropomyosins. As used herein, modified Pen a 1 peptides or Pen a 1 molecules with reduced or abolished IgE binding capacity are defined as any Pen a 1 molecule or peptide that contain one or more amino acid substitutions that reduce or abolish IgE binding of the peptide or Pen a 1 molecule.

In other preferred embodiments, the present invention provides for oral and/or immunotherapy using the modified Pen a 1 peptides or molecules, for example, a vaccine expressing the modified peptides or Pen a 1 molecules. In addition, modified tropomyosin used for the development of transgenic shrimp, crab, lobster, or crawfish in which the native tropomyosin production is reduced allowing for production of hypoallergenic seafood that will have reduced or abolished potential to induce allergic reactions.

Other aspects of the invention are disclosed *infra*.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the forty six, synthetic overlapping peptides spanning the entire sequence of Pen a 1 (length 15 amino acid residues, offset: 6 amino acids).

Figure 2 shows the results of the PepScan analysis of Pen a 1 peptides sera from 18 shrimp allergic subjects. (Peptide length: 15 amino acids, offset: 6 amino acids).

Figure 3 (3.1 - 3.5) shows the individually recognized epitopes and sequence comparison with allergenic and non-allergenic tropomyosins in the Pen a 1 regions 1-5.

Figure 4 shows the peptide amino acid sequence resulting from combinatorial substitutions and transfoming a Pen a 1 peptide into the homologous chicken tropomyosin (TM) sequence.

Figure 5 is an autoradiograph showing the reactivity of the combinatorial substituted peptides with IgE from individual sera..

Figure 6 illustrates the amino acid positions that are critical for binding to IgE.

Figure 7 shows the peptides recognized by the serum IgE of six shrimp allergic subjects, each spot representing a different peptide of all 46 tested.

Figure 8 shows the amino acid sequence comparison of Pen a 1 with other allergenic tropomyosins.

Figure 9 shows the sequence comparison of IgE-binding, recombinant peptides and non-IgE-binding synthetic peptides: Identical sequences are shaded.

Figure 10 shows 12 Pen a 1 varieties that contain substitutions in 78 positions and will reduce or abolish the IgE antibody reactivity of the Pen a 1 molecule.

DETAILED DESCRIPTION OF THE INVENTION

As discussed, the invention provides highly useful compositions and methods for modifying and particularly reducing a harmful or potentially harmful immune response against tropomyosin. Of interest are crustacea, arthropod, mollusk and arachnid tropomyosin, especially those of shrimp or insect origin.

The following definitions are used herein unless specified otherwise.

An antigen is a molecule that elicits production of an antibody such as an IgE antibody.

An antibody or other similar term refers to whole immunoglobulin as well as immunologically active fragments which bind antigen. The immunoglobulins and immunologically active fragments thereof include an antibody binding site. Exemplary antibody fragments include for example, Fab, F(v), Fab', F(ab')₂ fragments, "half molecules" derived by reducing the disulfide bonds of immunoglobulins, single chain immunoglobulins, or other suitable antigen binding fragments (see, e.g. Bird et al., *Science*, pp. 242-424 (1988); Huston et al., *PNAS (USA)*, 85:5879 (1988); Webber et al., *Mol. Immunol.*, 32:249 (1995)). The antibody or immunologically active fragment thereof, may be of animal (e.g. a rodent such as a mouse or rat), or chimeric form (see Morrison et al., *PNAS (USA)*, 81:6851 (1984); Jones et al., *Nature.*, 321:522 (1986)). Single chain antibodies of the invention can be preferred.

An allergan is a subset of antigens which elicits IgE production in addition to other isotypes of antibodies.

An allergic reaction is one that is IgE mediated with clinical symptoms primarily involving the cutaneous (uticaria, angiodema, pruritus), respiratory (wheezing, coughing, laryngeal edema, rhinorrhea, watery/itching eyes), gastrointestinal (vomiting, abdominal pain, diarrhea), and cardiovascular (if a systemic reaction occurs) systems.

An epitope is a binding site including an amino acid motif of between approximately five to fifteen amino acids which can be bound by an immunoglobulin. A linear epitope is one where the amino acids are recognized in the context of a simple linear sequence. A conformational epitope is one where the

amino acids are recognized in the context of a particular three dimensional structure.

An immunodominant epitope is one which is bound by antibody in a large percentage of the sensitized population or where the titer of the antibody is high, relative to the percentage or titer of antibody reaction to other epitopes present in the same protein.

A decreased allergic reaction is characterized by a decrease in clinical symptoms associated with exposure to an allergen, which can involve respiratory, gastrointestinal, skin, eyes, ears and mucosal surfaces in general.

The first step in making the modified allergan is to identify IgE epitope binding sites and/or immunodominant IgE binding sites. The second step is to mutate one or more of the IgE binding sites, preferably including at a minimum one of the immunodominant sites. The third step is to make sufficient amounts of allergan for administration to persons or animals in need of tolerance to the allergan, where the modified allergan is administered in a dosage and for a time to induce tolerance, or for diagnostic purposes. The modified allergan can be administered by injection, or in some cases, by ingestion or inhalation.

Allergans typically have both IgE and IgG binding sites and are recognized by T cells. The binding sites of the allergans can be identified using phage display libraries to identify conformational epitopes (Eichler and Houghten, (1995) *Molecular Medicine Today,* 1:174-180; Jensen-Jarolim et al., (1997) *J. Appl. Clin. Immunol.* 101:5153a) or by using defined peptides derived from the known amino acid sequence of the allergan (see examples below).

It is desirable to modify the IgE-binding epitopes of the allergan. In order to reduce or abolish the IgE reactivity, the individual peptides containing IgEbinding epitopes are mutated into the homologous sequences of the allergenic (invertebrate) to the vertebrate (non-allergenic) tropomyosins. The substitutions and their effect on the IgE-binding capability of modified Pen a 1 peptides can be categorized according to different criteria. First, the minimal number of substitutions that a peptide must carry to render it non-reactive and second, the maximal number of substitutions per peptide to allow the peptide to retain at least some IgE reactivity.

A modified allergan will typically be made usingrecombinant techniques. Expression in a prokaryotic or eukaryotic host including bacteria, yeast, and baculovirus systems are typically used to produce large (mg) quantities of the modified allergan.

A modified amino acid sequence, as used herein, refersto an amino acid sequence of tropomyosin which has been modified by substituting an amino acid such that modified amino acid sequence binding to an IgE molecule is reduced.

Substituted amino acids can be selected from a non-polar aliphatic group; a polar, uncharged group; an aromatic group; a positively charged group; or a negatively charged group. For representative amino acids from each group and a description of each group see Alberts et al; Molecular Biology of the Cell, Chapters 2 and 3, second edition, Eds. M. Robertson, R. Adams, Garland Publishing, NY.

Crustacea as used herein, refer to shellfish such as crab, shrimp, lobster, crawfish.

Crustacea tropomyosins used herein, refers to tropomyosin from shellfish as defined above.

Assays to assess an immunological change after the administration of the modified allergan are known to those skilled in the art. Standard IgE antibody tests include such conventional assays such as, for example, RAST (Sampson and Albergo, 1984), ELISAs (Burks et al., 1986) immunoblotting (Burks et al. 1988), and *in vivo* skin tests (Sampson and Albergo, 1984). Objective clinical symptoms can be monitored before and after the administration of the modified allergan to determine any change in clinical symptoms.

Reference herein to a "standard IgE antibody test" or related phrase means any one of the foregoing tests, particularly the IgE ELISA test, in which at least about 45%, preferably at least about 60%, more preferably at least about 75% of the binding between the crustacea tropomyosin and the IgE antibody has been reduced. Suitable controls for performing such a test are generally known in the field and include identified IgE antibodies that bind the tropomyosin and crustacea

tropomyosin e.g., that isolated from shrimp. More particular tropomyosins and IgE antibodies for performing the test are discussed below.

"Desensitization" as used herein is defined by a sufficient decrease in IgE antibodies, as measured by the above standard IgE tests, wherein the allergic reaction of an individual manifests a decrease in clinical symptoms associated with exposure to an allergen, which can involve respiratory, gastrointestinal, skin, eyes, ears and mucosal surfaces in general.

A desensitizing amount as used herein, is a therapeutic composition of the present invention employed in a physically discrete unit suitable as unitary dosages for a primate such as a human, each unit containing a predetermined quantity of active material calculated to produce the desired therapeuticeffect in association with the required diluent or carrier. Precise desensitizing amounts of the therapeutic composition to be administered will be guided by the judgment of the practitioner, however the unit dose will generally depend on the route of administration and be in the range of 10 ng/kg body weight to 50 mg/kg body weight per day, more typically in the range of 100 ng/kg body weight to about 10 mg/kg body weight per day.

Transgenic animals expressing the modified allergan have two purposes. First, they can be used as a source of modified allergan for use in immunotherapy and second, appropriately modified animals can be substituted for the original animal making immunotherapy unnecessary. Methods for the engineering of animals, for example, shrimp, crab, lobster or crawfish, are well known to those skilled in the art. See for example, A. Colman, "Production of Therapeutic Proteins in the Milk of Transgenic Livestock" (1988) *Biochem. Soc. Symp.* 63:141-147 and Colman, *Am. J. Clin. Nutr.* 63(4):639S-645S, the teachings of which are incorporated herein.

It is important to administer the modified allergan to an individual (human or animal) to decrease the clinical symptoms of allergic disease by using a method, dosage, and carrier which are effective. The modified allergan will typically be administered in an appropriate carrier such as saline or a phosphate saline buffer. The modified allergan can be administered by injection subcutaneously,

intramuscularly, or intraperitoneally, by aerosol, inhaled powder, as a suppository, or by ingestion.

While one or more modified allergans of the invention may be administered alone, they may also be present as part of a pharmaceutical composition in mixture with conventional excipient, preferably a pharmaceutically acceptable organic or inorganic carrier substances that is generally suitable for oral or nasal delivery. However, in some cases, other modes of administration may be indicated in which case the modified allergans, or combination of modified allergans thereof, can be combined with a vehicle suitable for parenteral, oral or other desired administration and which do not deleteriously react with the modified allergans and are not deleterious to the recipient thereof. Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohol, vegetable oils, polyethylene glycols, gelatin, lactose; amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethal fatty acid esters, hydroxymethyl-cellulose, polyvinylpyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired mixed with auxiliary agents, e.g. lubricants, preservatives, stabilizers, wetting agents, emulsifiers, slats for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously react with the modified allergans.

For parenteral application, particularly suitable are solutions, preferably oily or aqueous solutions as well as suspensions, emulsions, or implants, including suppositories. Ampoules are convenient unit dosages.

For enteral application, particularly suitable are tablets, dragees or capsules having talc and/or carbohydrate carrier binder or the like, the carrier preferably being lactose and/or corn starch and/or potato starch. A syrup, elixir or the like can be used wherein a sweetened vehicle is employed. Sustained release compositions can be formulated including those wherein the active component is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc.

It will be appreciated that the actual preferred amounts of modified allergans, or combination of modified allergans, used in a giventherapy will vary

according to the modified peptide or combination of peptides being utilized, the mode of application, the particular site of administration, etc. Optimal administration rates for a given protocol of administration can be readily ascertained by those skilled in the art.

The nucleotide molecule encoding the modified allergan can also be administered directly to the patient, for example, in a suitable expression vector such as a plasmid, which is injected directly into the muscle or dermis.

The modified allergan can be expressed by a vector containing a DNA segment encoding the modified allergan.

These can include vectors, liposomes, naked DNA, adjuvant-assisted DNA, gene gun, catheters, etc. Vectors include chemical conjugates such asdescribed in WO 93/04701, which has a targeting moiety (e.g. a ligand to a cellular surface receptor), and a nucleic acid binding moiety (e.g. polylysine), viral vector (e.g. a DNA or RNA viral vector), fusion proteins such as described in PCT/US95/02140(WO 95/22618) which is a fusion protein containing a target moiety (e.g. an antibody specific for a target cell) and a nucleic acid binding moiety (e.g. a protamine), plasmids, phage etc. The vectors can be chromosomal, nonchromosomal or synthetic.

Preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include moloney murine leukemia viruses. DNA viral vectors are preferred. Viral vectors can be chosen to introduce the modified allergan to cells of choice. Such vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as herpes simplex I virus (HSV) vector (Geller, A.I et al, J. Neurochem., 64:487(1995); Lim, F., et al., in DNA Cloning: Mammalian Systems, D. Glover, Ed. (Oxford Univ. Press, Oxford, England) (1995); Geller, A.I. et al., Proc. Natl. Acad. Sci. USA 87:1149 (1990)) Adenovirus vectors (LeGal LaSalle et al., Science, 259:988 (1993); Davidson, et al., Nat. Genet. 3:219 (1993); Yang et al., J. Virol. 69:2004 (1995)) and Adeno-associated virus vectors (Kaplitt, M.G. et al., Nat. Genet. 8:148 (1994)).

Pox viral vectors introduce the gene into the cells cytoplasm. Avipox virus vectors result in only short term expression of the nucleic acid. Adenovirus

vectors, adeno-associated virus vectors and herpes simplex virus vectors are preferred for introducing the nucleic acid into neural cells. The adenovirus vector results in a shorter term expression (about 2 months) than adeno-associated virus (about 4 months), which in turn is shorter than HSV vectors. The vectors can be introduced by standard techniques, e.g. infection, transfection, transduction or transformation. Examples of modes of gene transfer include for example, naked DNA calcium phosphate precipitation, DEAE dextran, electroporation, protoplast fusion, lipofection, cell microinjection and viral vectors.

The vector can be employed to target essentially any desired target cell. For example, stereotaxic injection can be used to direct the vectors (e.g. adenovirus, HSV) to a desired location. Other methods that can be used include catheters, intravenous, parenteral, intraperitoneal, and subcutaneous injection, and oral or other known routes of administration.

Another method is DNA immunization. DNA immunization employs the subcutaneous injection of a plasmid DNA (pDNA) vector encoding a specific allergenic protein. The pDNA sequence is taken up by antigen presenting cells (APC). Once inside the cell, the DNA encoding allergan is transcribed and translated. The allergan is then presumably presented on the surface of the APC in the context of the major histocompatibility complex (MHC) to T-cells. This endogenously produced allergenic protein or protein fragment induces a T_H1 phenotypic response with up-regulation of IFN-γ, an increase in IgG_2, and suppression of allergan-specific IgE production (Speigelberg HL, Orozco EM, Roman M, et al. *Allergy* 1997;52:964-70; Slater JE, Zhang YJ, Arthur-Smith A, et al., *J Allergy Clin Immunol.* 1997;99:S504). Oral delivery of DNA immunizations has also been described. Its been utilized as an immunoprophylactic strategy to modulate peanut antigen-induced anaphylaxis (Roy K, Mao HQ, Huang SK, Leong KW. *Nat. Med.* 1999;5(4):387-91). In this model, the oral delivery of DNA complexed to chitosan, a biocompatible polysaccharide, also favored a T_H1 response and suppressed the T_H2 allergic immune response.

The vector pDNA can also be conjugated to immunostimulatory sequences (ISS). These ISS contain unmethylated cytosine and guanine dinucleotide repeat motifs. These CpG motifs stimulate APCs and natural killer cells to secrete IFN-γ and IL-12, cytokines that promote immune deviation toward the T_H1 phenotype and

away from the allergic T_H2 phenotype (Chu RS, Targoni OS, Krieg AM, Lehman PV, Harding CV. *J. Exp. Med.* 1997;186:1623-31). These ISS stimulate immune deviation to the T_H1 phenotype when administered in several ways. They can be administered with DNA encoding the allergan (DNA immunization) (Hus C-H, Chua KY, Tao MH, Lai YL, Wu HD, Huang SK, et al., *Nat. Med.* 1996;2:540-4.), when given alone (Bohle B, Jahn-Schmid B, Maurer D, Kraft D, Ebner C. *Eur. J. Immunol.* 1999;29:2344-53), or when conjugated with allergan. Although the majority of these DNA immunization techniques have been studied in the mouse model, Tighe and colleagues describe enhanced immune deviation to the T_H1 phenotype and reduced allerganicity after injection immunotherapy with the major ragweed allergan, Amb a 1, conjugated to ISS in mice and rabbits, as well as in primates (Tighe H, Takabayashi K, Schwartz D, et al., *J. Allergy. Clin. Immunol.* 2000;106:124-34]).

A vaccine as used herein, can include any of the above viruses or vectors containing the entire nucleic acid sequence of the tropomyosin molecule, fragments thereof; modified nucleic acid sequences of the tropomyosin molecule; the entire amino acid sequence of the tropomyosin molecule or fragments thereof; modified amino acid fragments of the tropomyosin molecule or any peptides embodied in the invention.

The vaccine may be introduced in a suitable carrier. For example, sterile saline solution or sterile phosphate buffered saline.

In order to produce the desired therapeutic effect, as embodied in this invention, the vaccine is desirably administered bysubcutaneous or intramuscular injection. The treatment may consist of a single dose of vaccine or a plurality of doses over a period of time. An advantageous treatment schedule requires administration of two doses of vaccine with an interval of 3 to 7, preferably 4 to 6 weeks between doses. If longer protection is required, booster doses may be administered after longer intervals, for instance after 6 months or annually. Those who are skilled in the art may modify the vaccine regimen according to the individual patient.

It is recommended that each dose is 0.5 to 5 ml, preferably 1 to 3 ml, most preferably 2 ml of vaccine.

Antibodies of the invention can be prepared by techniques generally known in the art, and are typically generated to a purified crustacean tropomyosin molecule, to a modified crustacean tropomyosin molecule, preferably to peptides of a modified crustacean tropomyosin molecule or more preferably to modified peptide fragments thereof.

More particularly, antibodies can be prepared by immunizing a mammal with the above molecules, alone or complexed with a carrier. Suitable mammals include typical laboratory animals such as sheep, goats, rabbits, guinea pigs, rats and mice. Rats and mice, especially mice, are preferred for obtaining monoclonal antibodies. The antigen can be administered to the mammal by any number of suitable routes such as subcutaneous, intraperitoneal, intravenous, intramuscular or intracutaneous injection. The optimal immunizing interval, immunizing dose, etc. can vary within relatively wide ranges. Typical procedures involve injection of the antigen several times over a number of months. Antibodies are collected from serum of the immunized animal by standard techniques and screened to find antibodies specific for tropomyosin, modified tropomyosin, modified peptides of tropomyosin and fragments thereof. Monoclonal antibodies can be produced in cells which produce antibodies and those cells used to generate monoclonal antibodies by using standard fusion techniques for forming hybridoma cells. See G. Kohler, et al., *Nature,* 256:456 (1975). Typically this involves fusing an antibody producing cell with an immortal cell-line such as a myeloma cell to produce the hybrid cell. Alternatively, monoclonal antibodies can be produced from cells by the method of Huse et al., *Science,* 256:1275 (1989).

One suitable protocol provides for intraperitoneal immunization of a mouse with a composition comprising the above-discussed antigens, conducted over a period of about two to seven months. Spleen cells can then be removed from the immunized mouse. Serum from the immunized mouse is assayed for titers of antibodies specific for the tropomyosin antigen selected, prior to excision of spleen cells. The excised spleen cells are then fused to an appropriate homogenic or heterogenic (preferably homogenic) lymphoid cell line having a marker such as hypoxanthine-guanine phosphoribosyltransferase deficiency (HGPRT) or thymidine kinase deficiency (TK). Preferably a myeloma cell is employed as the lymphoid cell line. Myeloma cells and spleen cells are mixed together, e.g. at a ratio of about 1 to

4 myeloma cells to spleen cells. The cells can be fused by polyethylene glycol (PEG) method. See G. Kohler, et al., *Nature, supra*. The thus cloned hybridoma is grown in a culture medium, e.g. RPMI-1640. See E. More, et al., *J. Amer. Med. Association,* 199:549 (1967). Hybridomas grown after the fusion procedure are screened such as by radioimmunoassay or enzyme immunoassay for secretion of antibodies that bind to the above discussed tropomyosin antigens. Preferably an ELISA is employed for the screen. Hybridomas that show positive results upon such screening can be expanded and cloned by limiting dilution method. The isolated antibodies can be further purified by any suitable immunological technique including affinity chromatography.

Tropomyosin protein, including fragments and modified tropomyosins are often provide in substantially pure form. That is, the proteins have been isolated from cell substituents that naturally accompany it so that the proteins are present in at least 90 to 95% homogeneity (w/w). Proteins having at least 98 to 99% homogeneity (w/w) are most preferred for many pharmaceutical, clinical and research applications including the vaccines disclosed herein. Once substantially purified the protein should be substantially free of contaminants for therapeutic applications. Once purified partially or to substantial purity, the proteins can be used therapeutically, or in performing *in vitro* or *in vivo* assays as disclosed herein. Substantial purity can be determined by a variety of standard techniques such as chromatography and gel electrophoresis.

The tropomyosins in accord with the invention can be separated and purified by appropriate combination of known techniques. These methods include, for example, methods utilizing solubility such as salt precipitation and solvent precipitation, methods utilizing the difference in molecular weight such as dialysis, ultra-filtration, gel-filtration, and SDS-polyacrylamide gel electrophoresis, methods utilizing a difference in electrical charge such as ion-exchange column chromatography, methods utilizing specific affinity such as affinity chromatograph, methods utilizing a difference in hydrophobicity such as reverse-phase high performance liquid chromatograph and methods utilizing a difference in isoelectric point, such as isoelectric focusing electrophoresis, metal affinity columns such as Ni-NTA. See generally Sambrook et al. and Ausubel et al. supra for disclosure relating to these methods.

By the term specific binding or similar term is meant a molecule disclosed herein which binds another molecule, thereby forming a specific binding pair, but which does not recognize and bind to other molecules as determined by, e.g., Western blotting, ELISA, RIA, gel mobility shift assay, enzyme immunoassay, competitive assays, saturation assays or other suitable protein binding assays known in the field.

The modified tropomyosins of the invention can be made by one or a combination of strategies. For example, nucleotide sequences encoding tropomyosin of crustacea or anthrop origin can be altered by mutations such as substitutions, additions or deletions that provide for functionally equivalent nucleic acid sequence having reduced antigenicity (at the protein level). Preferred mutations are substitutions in the tropomyosin regions disclosed herein. In particular, a given nucleotide sequence can be mutated *in vitro* or *in vivo*, to create variations in coding regions and/or to form new restriction endonuclease sites or destroy preexisting ones and thereby to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used including, but not limited to, *in vitro* site-directed mutagenesis (Hutchinson et al., *J. Biol. Chem.*, 253:6551 (1978)), use of TAB Registered TM linkers (Pharmacia), PCR-directed mutagenesis, and the like. The functional equivalence of such mutagenized sequences, as compared with unmutagenized sequences, can be empirically determined by comparisons of structural and/or functional characteristics.

The present invention is further illustrated by the following Examples. These Examples are provided to aid in the understanding of the invention andare not construed as a limitation thereof.

EXAMPLES

Materials and Methods

Subjects' Sera

Sera collected from 18 atopic shrimp-allergic subjects were used to identify the IgE-binding regions of shrimp tropomyosin. All 18 subjects fulfilled four criteria: (1) history of respiratory (wheezing or shortness of breath), dermatologic (uticaria or angiodema), or gastrointestinal (nausea, vomiting, and/or diarrhea) symptoms occurring within 1 h following ingestion of shrimp; (2) positive immediate skin prick test (SPT, wheal >3mm) to the cooked brown shrimp *(Penaeus aztecus)*;

(3) elevated shrimp-specific IgE levels demonstrated by radioallergosorbent test (RAST, binding >3%) [24]; and (4) strong IgE reactivity to purified shrimp tropomyosin by immunoblot analysis. Three shrimp-allergic subjects without IgE reactivity to shrimp tropomyosin by immunoblot but fulfilling the other criteria for shrimp allergy were used as negative controls.

Shrimp extract and shrimp tropomyosin purification

Shrimp extract from locally purchased raw brown shrimp was prepared as described previously [24]. Pen a 1 was purified from shrimp extract by preparative SDS-PAGE (Model 491 PrepCell, Biorad). Briefly, shrimp extract was separated on the 28 mm ID column using Laemmli discontinuous SDSPAGE buffer system [25]. A 15 mm-high stacking gel (5%T, 1.5%C) poured on top of the 65 mm-high separation gel (11%T, 1.5%C) was used to separate shrimp proteins and the fractions containing Pen a 1 were collected and pooled.

IgE recognition of Pen a 1 by immunoblot analysis

Pen a 1 was run by SDS-PAGE [25] and transferred onto a CNBractivated [26] nitrocellulose membrane (0.45 µm, BAS 45, Schneider and Schuell, Germany) at 0.8 mA/cm^2 for 30 min by semi-dry blotting [27]. The blots were blocked in TBS-Tween for 30 min, dried and stored between filter paper until use. Seventy five µl of sera from each subject, diluted 1:2 in TBS-Tween (0.1M Tris HCl, pH 7.5, 0.1 M NaCl, 2.5 mM $MgCl_2$, 0.05% Tween) were incubated for 2 h with the Pen a 1-containing membrane using a Surf-blot apparatus (Idea Scientific, Minneapolis MN). The membranes were washed in TBS-Tween and dried. To visualize IgE-binding proteins, the blot was incubated for 2 h with alkaline phosphatase conjugated, monoclonal mouse anti-human IgE (Southern Biotechnology Associates, Birmingham AL) diluted 1:1000 in TBSTween and washed 3 times for 10 min in TBS-Tween. For detection of bound IgE, the membrane was washed 5 min in 37°C warm TBS-AP (0.1 M Tris-HCl, 0.1 M NaCl, 5 mM $MgCl_2$, pH 9.5) and antibody binding was visualized at using the substrate/chromogen mixture for alkaline phosphatase at 37°C containing 450 µM 5-bromo-4-chloro-indolyl-phosphate disodium salt (BCIP; Sigma) and 400 µM nitroblue tetrazolium chloride (NBT; Sigma) solubilized in TBS-AP [28]. The reaction was stopped with deionized water and the blots were dried.

IgE antib dy reactivity to synthetic verlapping Pen a 1 peptides: Peptide

synth sis and IgE-binding assay

Forty-six overlapping peptides were synthesized spanning the entire 284 amino acid residue length of Pen a 1. Each peptide had a length of 15 amino acid residues with an offset of six in relation to the previous and following peptides with the exception of peptide that had an offset of five amino acid residues (Figure 1).

Overlapping peptides were synthesized with Fluorenylmethoxycarboyl (Fmoc) amino acids on cellulose membranes containing free hydroxyl groups according to the manufacturer's instructions of the SPOTS Epitope Mapping System (Genosys Biotechnologies, The Woodlands, TX). For the preparation of the Fmoc-amino acid active ester solutions, each amino acid was dissolved in purified 1-methyl-2 pyrrolidinone (NMP 99%, Sigma-Aldrich, St. Louis, MO). Each amino acid solution was aliquoted and stored at -20°C until ready for use. Due to intrinsic instability, arginine was dissolved in NMP immediately prior to each synthesis cycle.

The procedure for peptide synthesis was performed as described previously [29-31]. Briefly, each cycle began by sterification of an Fmoc amino acid to the SPOTs cellulose membrane (Genosys Biotechnologies, Inc., The Woodlands, TX). Following incubation, the membranes were washed in N,N-didethylformamide (DMF, EM Science, Gibbstown, NJ) and 4% acetic anhydride in DMF was added to acetylate and block any uncoupled amino groups to prevent further reaction of these groups and formation of deletion sequences. After acetylation protective Fmoc groups were cleaved by incubation in 20% piperidine (Aldrich chemicals, Milwaukee, WI) in DMF, to render nascent peptides reactive. Each additional Fmoc amino acid was esterified to the previous one by the same process until the desired peptide is generated. After addition of the last amino acid, the protecting groups on the side chains of the amino acids were removed using a 1:1:0.05 mixture of dichloromethane (Aldrich)/ trifluoroacetic acid (TFA, Aldrich)/ triisobutylsilane (Aldrich), followed by washing in methanol. Membranes were either probed immediately or dried and stored at -20°C until needed.

For the IgE-binding assay, the membranes were first rinsed in methanol and washed in Tris buffered saline (TBS, pH 7.5) 3 times for 10min. The membranes were incubated in blocking solution (Genosys) diluted 1:10 in TBS for 2 h and then overnight with the patient's serum diluted 1:5 with blocking buffer. After washing three times for 15 min in TBS-Tween (TBS, 0.5% Tween; pH 7.5), IgE reactivities

were detected using 0.8 µCi per membrane of ^{125}I-labeled horse-anti human IgE (Sanofi Diagnostics Pasteur, Chasca, MN) diluted 1:10 in Genosys blocking solution. Next day, the membranes were washed 3 times for 15 min in TBSTween and placed between plastic sheets and exposed to X-ray film for 72 hours.

For interpretation of results, IgE reactivities were graded according to their intensity into four categories: negative (0), weak (1), medium (2) and strong (3), and color-coded as follows: negative (white), weak intensity (light gray), medium intensity (dark gray), and strong intensity (black). The intensity of the IgE reactivities was determined visually by agreement of 3 different investigators, who graded the reactivities independently assigning the above scores.

Example 1

IgE binding regions of Pen a 1

Table 1 shows the frequency and intensity of the IgE-recognition of the different peptides by the 18 subject's sera. A wide range of peptides (from 1 to 16, mean of 8 per subject) were recognized by serum IgE of the shrimp-allergic, Pen a 1-reactive sera. In contrast, none of the three control sera from shrimp-allergic, Pen a 1-non reactive subjects showed IgE binding to any of the 46 peptides tested (data not shown). As an example, Figure 7 shows the peptides recognized by the serum IgE of 6 shrimp-allergic subjects, with each spot representing a different peptide of all 46 tested. The number of subjects who recognized an individual peptide varied from 0 (0%) to 13 (72.2%). IgE-binding peptides were detected over most of the tropomyosin molecule. An intensity score (0-3) for each peptide was calculated by adding the individual scores obtained with the different sera (peptide score). The mean intensity score of all peptides was obtained by adding all the peptide scores and dividing by 46 peptides (mean peptide score). A major IgE binding region was defined as a region recognized by serum IgE from more than 9/18 (50%) of the subjects, and/or when the score for intensity of IgE-binding of a particular peptide was larger than mean + 1 SDEV the mean peptide score (5.9 + 6.1).

Based on frequency and intensity of the IgE reactivities, five major IgE binding regions were identified. All five major IgE-binding regions spanned from 1 to 4 peptides, with a length from 15 to 38 amino acid residues. Major IgE-binding regions identified were, region 1: Pen a 1 (43-57), region 2: Pen a 1 (85-105), region 3: Pen a 1 (133-148), region 4: Pen a 1 (187-202) and region 5: Pen a 1 (247-284).

Region 1 is recognized by 10/18 (55.5%) subjects, region 2 by 15/18 (83.3%), region 3 by 10/18 (55.5%), region 4 by 5/18 (27.5%) and region 5 by 12/18 (66.6%). The score for intensity of IgE recognition was 18, 19, 20, 12, and 12.2 for the five regions, respectively.

Example 2

Identified IgE-binding epitopes and epitope cores

Within regions 1, 2, 3 4 and 5 IgE-binding epitopes were identified using 5 to 9 amino acids-long peptides with an offset of two amino acids. If no or only minimal reactivity was detected the peptide size was increased to 9 to 15 amino acid residues. The peptides that were found to be IgE-binding within regions 1 to 5 are also summarized in table 2.

Figures 3.1 to 3.5 show the identified strongest individual IgE-binding reactivities (epitopes) of regions 1 to 5, common epitope cores, and sequence comparisons with allergenic and non-allergenic tropomyosins (Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Metapenaeus ensis* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMs), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Insecta: *Periplaneta americana* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus, D. farinae* (house dust mites, Der p 10, Der f 10). Vertebrata: *Gallus gallus* (chicken, alpha-tropomyosin GalgαTM; beta-tropomyosin GalgβTM), *Sus scrofa* (pig, SussβTM), *Salmo trutta* (Atlantic salmon, SaltTM), *Oryctolagus cuniculus* (rabbit, OrycβTM) (GenBank data)).

Region 1

All four subjects tested with peptides in region 1. For all for subjects the epitope was identical (Pen a 1 43-55, VHNLQKRMQQLEN) and 14 amino acids long. This sequence is identical to those from Met e 1, Hom a 1, and Pan s 1. However, slow lobster tropomyosin (HomaTMs) differs in 7 positions and vertebrate tropomyosins differ in up to nine positions.

Region 2

Three of the four subjects tested reacted with Pen a 1 87-101 (ALNRRIQLLEEDLER), one subject reacted with a fraction of this sequence, Pen a 1 91-101 (RIQLLEEDLER), which is effectively the core of this epitope. This core

sequence is identical with those of other Crustacea and Per a 7, whereas Der p 10 carries one substitution in position 95. All vertebrate tropomyosins carry the same three substitutions in positions 95, 98, and 100.

Region 3

Six out of seven subjects tested reacted with peptides in region 3 (Pen a 1 133-146). The size of the individual epitope ranged from 6 to 9 amino acid residues. The core epitope of region 3 was Pen a 1 137-141 (DEERM) which is recognized by all 6 subjects and is identical to the homologous sequences of American cockroach (*Periplaneta americana*) and house dust mite (*Dermatophagoides pteronyssinus*) allergenic tropomyosins, Per a 1 and Der p 10, respectively. The comparison with vertebrate tropomyosin shows a substitution in position 141 where an arginine (R) is substituted for a lysine (K) residue.

Region 4

All individual epitopes of the three subjects tested varied in length from 11 to 15 amino acid residues and begin with position 187. The shortest of the three epitopes (ESKIVELEEEL) is considered to be the epitope core. All homologous sequences of invertebrate tropomyosins are identical to the Pen a 1 sequence with the exception of Pan s 1 that carries a substitution in position 190. The vertebrate tropomyosins differ from the homologous Pen a 1 sequence in up to four positions.

Region 5

In region 5 (Pen a 1 247-284) three epitopes were identified. All four subjects reacted with Pen a 1 266-273 (KYKSITDE). This sequence was for all four subjects the minimal IgE-binding site; no other peptide showed stronger reactivity. This epitope differs from both allergenic, arthropod and non-allergenic, vertebrate tropomyosins. The second epitope of region 5, is centered around a core, Pen a 1 251-259 and is recognized by 3/4 subjects; the core does not differ for the homologous sequences of Per a 1 or Der p 10 but differs in three positions from non-allergenic, vertebrate tropomyosins. Two of 3 individually recognized epitopes were larger (12 residues) than the average epitope size (9 residues). The third epitope in region 5 (Pen a 1 273-281) is only recognized by one subject and does not seem to be an important epitope.

The Examples shown above demonstrate that individual epitopes have large

overlaps and are dustered around a common core sequences. The identity of some of these epitopes cores with homologous, allergenic tropomyosins of American cockroach (*P. americana*) and house dust mite (*D. pteronyssinus*) tropomyosin may explain reported cross-reactivities between shrimp and other arthropods. The limited amino acid sequence differences between individual epitopes as well as epitope cores and non-allergenic tropomyosins makes it possible to test all possible combinations of amino acid substitutions (see below).

Example 3

Combinatorial Substitution Analysis

In order to reduce/abolish the antibody reactivity, the individual IgE-binding peptides (= epitopes) of regions 1, 2, 3, and 4 are gradually mutated into the homologous sequences of invertebrate (allergenic) and vertebrate (non-allergenic) tropomyosins (figure 4). Individual sera were tested for IgE reactivities to these peptides. The reactivities were scored according to their intensities and color-coded (minimal, moderate, [illegible], maximal). The sequences of and the reactivities to these peptides are listed in the following tables 3.1, 3.2., 3.3., 3.5, and 3.5. The corresponding autoradiographs are shown in figures 5.1, 5.2., 5.3., and 5.4, respectively.

The aim of these experiments was to study the effects of amino acid substitutions on the IgE binding capacity of an allergenic epitope if a nonallergenic homologous protein is not known, homologous sequences of allergenic, invertebrate and non-allergenic, vertebrate tropomyosins served as templates for the substitutions. The following muscle tropomyosins were used as templates (GenBank data):

Crustacea:	*Homarus americanus* (Atlantic lobster, Hom a 1)
	H. americanus slow muscle tropomyosin (HomaTMs)
	Panulirus stimpsoni (spiny lobster, Pan s 1)
Insecta:	*Periplaneta americana* (American cockroach, Per a 7)
Arachnida:	*Dermatophagoides pteronyssinus*, (house dust mite, Der p 10).
	D. farinae (house dust mite, Der f 10)
Vertebrata:	*Gallus gallus* (chicken alpha-tropomyosin, GalgαTM)
	G. gallus (chicken beta-tropomyosin, chicken GalgβTM),
	Sus scrofa (swine alpha tropomyosin, SusαTM),
	Salmo trutta (Atlantic salmon, SaltTM1, SaltTM2)

Oryctolagus cuniculus (rabbit alpha-tropomyosin, OryαTM)
O. cuniculus (rabbit beta-tropomyosin, OryβTM)

Due to the limited number of possible substitutions, the combinatorial substitution analysis was performed with individual sera rather than serum pool. The substitutions and their effect on the IgE-binding capability of modified Pen a 1 peptides can be categorized according to different criteria. First, the minimal number of substitutions that a peptide must carry to render it non-reactive and second, the maximal number of substitutions per peptide allow the peptide to retain at least some IgE reactivity. In all 17 epitope-subject combinations that were studied by mutational analysis, one substitution may be sufficient to render Pen a 1 epitopes non-IgE binding. However, this is the case in only 57.6% of the 170 peptides tested that carried a single substitution; the remaining 424% of the peptides carrying a single substitution still bind IgE antibodies. 82.4% of peptides that differ from the homologous Pen a 1 sequence in two positions are still able to bind IgE antibodies. In general for most (99.9%) peptides that carry threeor more substitutions do not show any IgE antibody reactivity; only one peptide carrying three substitutions bond IgE antibodies.

Critical Amino Acid Positions

The mutated peptides were analyzed in regard to positions within individual epitopes that were "critical" for IgE antibody binding. A critical position was defined as a position that, when substituted, abolished the IgE antibody reactivity of all peptides that contained this mutation. The results of this analysis is summarized in figure 6; it shows the critical amino acid positions (marked in green)for each individually epitopes within regions 1, 2, 3, and 5. Furthermore, figure 6 gives the amino acid that has to be substituted into these critical positions to abolish IgE antibody binding.

Table 5 lists all amino acid substitutions in Pen a 1 epitopes 1, 2, 3a, 3b, 5a, 5b, and 5c that result in complete loss or reduction of IgE antibody reactivity. The first column lists all the sequences that carry substitutions that abolished IgE antibody reactivity with any of the sera that were used to analyze that particular epitope. The second column lists all the sequences that carry substitutions which in some cases reduce or abolish IgE antibody reactivity with the sera that were used to analyze the previously unaltered Pen a 1 epitopes. The sequences are

named according to the substitutions they carry (King et al., Allergan Nomenclature. WHO/IUIS Allergan Nomenclature Subcommittee *Int. Arch. Allergy Immunol.* 1994, 105(3):224-233). For example, Pen a 1 44 is a Pen a 1 sequence that has an I (Isoleucine) in Pen a 1 position 44. This Isoleucine replaces a H (Histidine) that can be found in position 44 in the unaltered Pen a 1 sequence (see row labeled as "Pen a 1 sequence").

Critical Amino Acid Positions of Epitopes of Region 1

No critical amino acid position was identified within epitopes of region 1.

Critical Amino Acid Positions of Epitopes of Region 2

Within the epitopes of region 2, three critical amino acid positions (95, 98, 100) were identified. Of these three positions, position 95 seems to be the most crucial, since a substitution of Leucine (L) with Phenylalanine (F) abolished the IgE antibody reactivity of all three subjects' sera tested.

Critical Amino Acid Positions of Epitopes of Region 3

To Abolish the IgE antibody reactivity of individual 3a epitopes, mutations in two critical positions are necessary. The first critical position is located at position 142 and an Aspartate (D) is replaced with Glutamate (E); it abolished the IgE antibody reactivity of four of the five epitope 3a-reactive sera. The second critical position is position 136; a Serine (S) has to be replaced with a Lysine (K) to abolish the IgE antibody reactivity of three subjects' sera.

The critical position of epitope 3b is position 144; a mutation from a Leucine (L) to a Glutamine (Q) abolished the IgE antibody reactivity of both subjects' sera tested.

Critical Amino Acid Positions of Epitopes of Region 5

Within the 5a epitopes, four critical amino acid positions (250, 252, 255, 260) were identified. Of these four positions, position 255 seems to be the most crucial, since a substitution of Arginine (R) with Aspartate (D) abolished the IgE antibody reactivity of all four subjects' sera tested. Similarly, a substitution (Serine (S) in Phenylalanine (F)) in the one critical position 269 of epitope 5b rendered all peptides containing this substitution non-IgE-binding. Epitope 5c had four critical positions (277, 278, 280, 281); however, since only one subject reacted tothis

epitope, it is not possible to identify the most crucial substitution.

Example 4

Amino acid compositi n of the IgE-binding regions

To determine if certain groups of amino acids may be important for IgE binding, the relative frequency of different amino acids in the tropomyosin molecule relative to the main IgE-binding regions were analyzed. Five categories of amino acids were considered: non-polar, aliphatic (alanine A, glycine G, isoleucine I, leucine L, proline P, valine V; polar, uncharged (cysteine C, asparagine N, methionine M, glutamine Q, serine S, threonine T; aromatic (phenylalanine F, tryptophane W, tyrosine Y); positively charged (histidine H, lysine K, arginine R) and negatively charged (aspartic acid D, glutamic acid E). The frequency of the different groups of amino acids in each IgE-binding region was considered in relation to the number of amino acids present in that group, supposedly that all amino acids have the same probability to appear in a protein (probability 1).For the whole molecule, negatively charged amino acids are 2.5 times more frequent than would be expected by chance. Contrarily, aromatic residues are almost absent in the tropomyosin molecule. The other three groups of amino acids are present in the molecule with the frequency expected considering all 20 amino acids equally probable. In the five main IgE-binding regions the frequency of each amino acid group is the same as the frequency observed in the whole molecule. No substantial differences in amino acid group composition in the five IgE-binding regions compared to the whole molecule were detected.

When individual amino acids were analyzed, it was noted that several amino acids such as proline, cysteine and tryptophane were absent from the molecule and others such as glycine, isoleucine, tyrosine and histidine were rarely found. In contrast, alanine, leucine, lysine, arginine, and glutamic acid were present in the molecule at least 2 times more frequent than expected by chance. Only alanine seems to be less frequent in the IgE-binding regions than in the rest of the molecule. No differences in respect to the distribution of amino acids in the tropomyosin molecule were noticed, since all other amino acids are similarly represented in Pen a 1.

Example 5

Allergenic motifs

In previous studies, the presence of tandem amino acid repeats contained within allergans has been reported [32, 33]. In our study, all five major IgE-binding regions identified within Pen a 1 contain the amino acid sequence LEXXL, where L is Leucine and X is usually a negatively charged amino acid such as glutamic acid (E) or aspartic acid (D). In regions 1 and 3, X may be an aspartic acid (D), glutamine (Q) or asparagine (N). The tandem motifs occupy positions 5357 (motif 1), 95-99 (motif 2), 144-148 (motif 3), 193-197 (motif 4) and 256-260 (motif 5). Even though IgE reactivities were detected to Pen a 1 peptides which did not contain the tandem repeat, every tandem amino acid repeat was found to be included within a major Pen a 1 IgE-binding region.

Example 6

Sequence identity and similarity of Pen a 1 and Pen a 1 IgE-binding regions with homologous regions in other allergenic and nonallergenic tropomyosins

Sequence identities (% of amino acids identical in both compared tropomyosins) and similarities (% of amino acids identical or belonging to the same amino acid group) in the amino acid sequences of different tropomyosins with Pen a 1 IgE-binding regions are represented in Table 4. Sequence identity among Pen a 1 and other tropomyosins varied from 56% (rabbit tropomyosin) to 98% (lobster fast tropomyosin); similarity in amino acid composition ranged from 72% to 98% respectively, with the highest similarities (over 80%) being observed within arthropods (insects, arachnids and crustaceans). Remarkably amino acid sequences of several IgE binding regions of Pen a 1 showed significant degree of identity with homologous sequences of other tropomyosins, specially among arthropods such as lobster, house dust mites, cockroach, fruit fly, reaching up to 100% identity in particular sequences (Table 4).

Similarities of Pen a 1 regions with tropomyosin of mollusks such as mussels, helminths such as Onchocerca, and surprisingly, also with vertebrates such as rabbit, although varied depending on the regions considered, were as high as 86%, 94% and 85% respectively. Region 1 is identical within crustaceans but differs from other invertebrates and vertebrates, with only 26%,60% or 33% identity with Schistosoma, insects and vertebrates respectively. Interestingly, regions 2 and 4 show 100% similarity with homologous regions of arthropod tropomyosins from American cockroach *Periplaneta americana* (Per a 7), fruit fly *Drosophila melanogaster* (Dro m TM), house dust mites *Dermatophagoides pteronyssinus* and

D. farinae (Der p 10, Der f 10). Furthermore, regions 2 and 4 show significant similarity with corresponding sequences in other tropomyosins, including those from vertebrates (up to 85 %). Regions 3 and 5 are identical within crustaceans, and the identity with tropomyosins of other arthropods reaches up to 89%. In contrast, these Pen a 1 regions differ substantially from those of homologous sequences (as low as 40% identity) in helminths and vertebrate tropomyosins.

Example 7

Comparison of IgE-binding regions identified in Pen a 1 and in other allergenic tropomyosins.

Even though many invertebrate tropomyosins have been identified as allergenic, the location of the IgE-binding epitopes has been partially identified for only a small number of them. Figure 8 shows the amino acid sequence comparison of Pen a 1 with other allergenic tropomyosins, whose B cell epitopes have been partially characterized such as Pen i 1 from the shrimp *Penaeus indicus*, Tur c 1 from the snail *Turbo cornutus*, and Cra g 1 from the oyster *Crassotrea gigas*. Results from a previous study by our group of IgE binding epitopes from Pen a 1 using a recombinant peptide library of Pen a 1 are also included (regions E2, E3, E4 and E6). Most of the 5 IgE binding regions identified in Pen a 1 in the current study partially or completely overlap with those observed in other allergenic tropomyosins. Of particular importance seems to be region 5, identified also as an important IgE-binding region in the snail tropomyosin, Tur c 1, since the strongest IgE reactivities of both shrimp-allergic and snail-allergic individuals were directed towards this region.

Over the last years, the primary structures of a number of allergans have been characterized and T and B cell epitopes of several allergans have been identified. Knowledge of the B-cell and T-cell epitopes in food allergans is important because this could serve as the basis for the development of new safer peptides for food allergy immunotherapy, as well as for the introduction of new genetically modified hypoallergenic foods. Although not yet fully established, immunotherapy with T-cell peptides of some allergans has been demonstrated in a few cases to be effective. For instance, peptide fragments containing T-cell epitopes of bee venom phospholipase A2 intact, whose B-cell epitopes have been modified to abolish IgE binding, have been successfully used for immunotherapy with a lower risk of reactions [34]. Therefore, identification and subsequent modification of the B-cell

epitopes of tropomyosin could serve as the basis for the development of new safer immunotherapy for food allergy, and as well as for the introduction of new hypoallergenic foods.

In this study, five major Pen a 1 IgE-binding regions have been identified using synthetic overlapping peptides, 15 amino acids long with offset of 6, spanning the whole length of Pen a 1. The five IgE-binding regions are distributed along the molecule at approximately every 42 amino acid residues. This results suggest a relation with the heptameric repeat pattern characteristic for the - helical, coiled-coil structure of tropomyosin [21]. The five regions identified contain at least 15 amino acid residues (region 5 spans 37 residues). Since other studies have determined the IgE binding epitopes of other allergans to consist of approximately 8 amino acids [29-31, 33], most probably the identified IgE-binding regions of Pen a 1 are larger than their epitopes and each region may contain several epitopes. The smallest IgE-binding sequences within these regions of Pen a 1 remain to be investigated.

Previous studies of the homologous cockroach allergans Per a 1 and Bla g 1 [32] and the latex allergan Hev b 5 [33], have reported the presence of tandem amino acid repeats within allergans. Per a 1 and Bla g 1 sequences contain multiple tandem amino acid repeats of approximately 100 amino acid residues.Hev b 5, presents 9 repeated amino acid sequences of the type XEEX or XEEEX; four peptides of 8 amino acids containing the sequence XEEX were found to be IgE binding epitopes. In contrast, none of the peptides containing the motif XEEEX bound IgE from latex-allergic subjects. In our study, all five major IgE-binding regions identified within Pen a 1 contain the amino acid sequence LEXXL. Even though IgE reactivity to other Pen a 1 peptides that do not contain the tandem repeat was detected, every repetition of the tandem motif was found to be included within a major Pen a 1 IgE-binding region.

Shrimp-allergic patients may react to other crustaceans and sometimes to mollusks [24, 35], and substantial in vitro cross-reactivity among crustaceans has been demonstrated. Furthermore, allergenic cross-reactivity between arachnids (house dust mite), crustaceans (shrimp) and mollusks (snails, limpet) has been suggested to be of clinical relevance, especially in subjects receiving mite immunotherapy [17, 19]. Also, it has been reported that some patients may become

mite and/or cockroach-allergic subsequently to their sensitization to crustacean tropomyosin due to unusual ingestion of crabs and shrimp.

Comparison of the amino acid sequence of the entire Pen a 1 molecule with different tropomyosins showed that the identity ranged from 56% (rabbit tropomyosin) to 98% (lobster fast muscle tropomyosin). Similarities were even higher when conservative substitutions were not considered as different amino acids; the highest similarities were found among arthropods which reached over 80%. When the amino acid sequences within Pen a 1 IgE binding regions where compared with corresponding areas in other tropomyosins, the degree of similarity was found to be remarkably high; 100% identity of all five regions with the major lobster allergan Hom a 1 (fast muscle tropomyosin) was detected explaining the high degree of cross-reactivity among crustaceans. Sequence identity of Pen a 1 IgE binding regions with Per a 7 and Der p 10 is very high (60-100%) which might suggest similar IgE-binding epitopes in arthropods. Also consistent with this observation is the identity of Pen a 1 regions 2 and 4 with homologous amino acid sequences of tropomyosin from *D. melanogaster*. Similarities of Pen a 1 regions with those from mollusks and helminths are lower, but still as high as 86 and 94%, respectively. The importance of the cross-reactivity with helminths has been far less studied, but also reported [36].

Furthermore, comparison of IgE-binding regions identified in Pen a 1 and in other allergenic tropomyosins support our results. Results from a previous study by our group of IgE binding epitopes from Pen a 1 using a recombinant library are also included (regions E2 [167-179], E3 [136-148], E4 [262-282] and E6 [157-169]) [37]. Most of the five IgE-binding regions identified in Pen a 1 in the current study partially or completely overlap with those observed in other allergenic tropomyosins. Regions 2 and 5 seem to be of particular importance, since the homologous sequences in oyster (Cra g 1) and in the snail (Tur c 1) tropomyosins bind IgE antibodies of mollusk-allergic subjects, thus supporting the notion that tropomyosin is the cause of clinically relevant cross-sensitization between crustaceans and mollusks [24, 35].

The Examples further show *in vitro* cross-reactivity among tropomyosins from different invertebrates on the molecular level and demonstrates the importance of protein structure and its relationship to their allerganicity.

Example 8

Characterization and Identificati n of Allergan Epitopes: R combinant P ptide Libraries and Synthetic, Overlapping Peptid s.

Material and Methods

Recombinant Peptide Library

In order to characterize Pen a 1 epitopes, a recombinant peptide library (Novatope epitope mapping system, Novagen) was constructed. The Pen a 1-coding plasmid was randomly cleaved by DNase I in the presence of Mn^{2+}, causing double strand cleavage. Electrophoretically separated fragments, averaging 50 to 150 bp in size, were eluted (QIAEX II Agarose Gel Extraction Kit, Qiagen), treated successively with T4 DNA polymerase and Tth DNA polymerase, ligated into the pTOPE T vector, and transfected into NovaBlue (DE3) cells. The library was screened with a sera pool of shrimp-allergic subjects and positive clones sequenced.

Synthetic, Overlapping Peptides

Forty-six overlapping peptides (length: 15 amino acids, offset: six amino acids, figure 1) spanning the entire Pen a 1 molecule, were synthesized using the SPOTs system (Genosys, The Woodlands, TX).The SPOTs system cellulose membrane contains 96 blue spots which are derivatized with a dimer of b-alanine-NH2 groups that provide six atom linkers (anchor) between the membrane and the peptide. Fmoc-OPfp esters of the amino acids are coupled by repeated amino acid pipetting and washing. Amino acids are linked together by a condensation reaction between the C-terminal and N-terminal groups of two amino acids in a C-terminal to N-terminal direction from the membrane. The coupling reaction is monitored visually by staining the free amines after each coupling cycle with bromophenol blue. The resulting peptides are covalently bound to the membrane at their C-terminus. Each synthesis cycle begins with esterifying the appropriate Fmoc amino acid to the cellulose membrane or the previous amino acid. The coupling reactions are followed by acetylation with acetic anhydride in N,N-dimethylformamide to render the peptides unreactive during subsequent cycles. The Fmoc protective groups are removed by adding piperidine to activate the nascent peptides. To add the remaining amino acids the same cycle of coupling, blocking, and deprotection is repeated until the desired peptides are generated. The side chains are then deprotected with a 20:20:1 mixture of dichloromethane, trifluoroacetic acid, and

triisobutylsilane and washed with methanol. The membranes are stored at-20°C until used. The synthesis schedules can be calculated using the software provided by Genosys or using the graphing calculator HP48GX. The advantage of the HP48GX printouts is that the positions of a particular amino acid are provided as graphs rather than lists of position numbers.

For immunodetection, the membranes were blocked and incubated with 1:5 diluted serum pool or individual sera of shrimp-allergic subjects overnight. IgE reactivities were detected, either using 10 ml ^{125}I-labeled horse-anti-IgE (0.08 μCi/ml; Sanofi Diagnostics Pasteur, Inc.) or monoclonal alkaline phosphatase-labeled anti-human-IgE (Southern Biotechnology Associates, Birmingham, AL, USA) and autoradiography. The exposure time for ^{125}I-labeled anti-IgE was 72 h. For the detection of IgE antibodies using the alkaline phosphatase-conjugated monoclonal antibodies blots were washed with freshly prepared assay buffer (100 mM diethanolamine/HCI, 1.0 mM $MgCl_2$ pH 10.0), incubated in 1:50 diluted Nitroblock®chemiluminescence enhancer (Tropix, Bedford, MA) for 5 min and incubated in a 1:1000 dilution of CSPD (disodium 3-(4-methoxy-spiro{dioxetane-3, 2'-(5'chloro) tricyclo[3.3.1.1.^{3,7}]decan}-4-yl)phenyl phosphate; Tropix) for 5 min. Excessive liquid was drained, and the blots were sealed between transparencies and exposed to autoradiography film for 15, 30, 60 and 120 sec.

Comparison IgE reactivities to Recombinant and Synthetic, Overlapping Peptides

Four recombinant peptides and nine synthetic peptides bound Pen a 1-specific IgE, respectively (Table 6) and are located at the N-terminus, center, and C-terminus of the Pen a 1 molecule. No reactivity was detected in the N-terminal part of Pen a 1 using the recombinant peptide library. In general, the SPOTs system detects more IgE-binding sequences. However, two IgE-binding sequences, Pen a 1 157-169 and Pen a 1 167-179 that were detected using the recombinant peptide library were not detected using synthetic, overlapping peptides even though the entire sequence Pen a 1 157-169 is part of a synthetic peptide. In contrast Pen a 1 167-179 is part of two overlapping peptides (figure 9).

Table 7 shows all the substitutions (position, pos; substituting amino acid, aa) that can be considered to reduce or abolish the IgE reactivity of major and minor IgE-binding regions. In addition to the five major regions, minor IgE-reactive

regions, defined as regions to which at least one allergic subject shows strong IgE antibody reactivity.

The Example shows that the methods may be used to identify IgE-binding sequences of food allergans, and the SPOTs procedure resulted in the identification of more epitopes of the major shrimp allergan Pen a 1. However, since the sequences of the synthetic, overlapping peptides have a defined offset, epitopes that are located on two peptides overlapping may not always be readilyfound. In regard to IgE detection, the usage of ^{125}I-labeled detection antibody seems to be superior over enzyme-labeled anti IgE antibodies. The regeneration of SPOTs membranes is possible, but it is prudent to test regenerated membranes for residual activity. We synthesize new peptides for each experiment. Besides the technical aspects other factors may influence the technique used for epitope identification of a given food allergan. First, to create a recombinant peptide library, it is necessary to have an expressed full-length allergan or fragments that span the entire length of the allergan and have significant overlaps. The sequences for synthetic, overlapping peptides can be deduced from information available through data bases such as GenBank or SwissProt. Second, its is not possible to ensure that entire allergan sequence is represented in the peptide library whereas overlapping peptides guarantee systematic coverage of the entire allergan sequence. Third, an advantage of the recombinant library method is that the peptide length is not limited to 15 residues as it is the case for the SPOTs system which may allow the identification of at least some conformational epitopes. An additional advantage of the Novatope system that it is easy to test additional patients' sera by simply growing more peptide-expressing *E.coli* and use lysates in a dot blot or grid blot. Synthetic peptides have to be resynthesized which requires in comparison a much higher experimental effort. Fifth, a major advantage of synthetic peptides is the ease in which the impact of amino acid substitutions have on the IgE binding of epitopes [38,39,40]; the side-by-side comparison of unmodified and mutated epitopes allows an easy quantification of changes of protein structure on the alleganicity of proteins [39]. This approach may be used to produce foods and other allergans with reduced allerganicity.

Since the synthesis conditions are not optimized for each amino acid or peptide the question arises whether the synthesized peptides have the correct sequence. The synthesis protocol uses an acetylation step at the end of each cycle

to acetylate any unreacted free amines with acetic anhydride. This prevents them from coupling to any subsequent amino acids and virtually eliminates the synthsis of deletion sequences. The purity of the peptides synthesized varies for each peptide and is dependent upon sequence and length even though the peptide purity is typically larger than 70% (Genosys, personal communication). As a consequence it is essential to verify the results obtained with overlapping peptides with highly purified peptides when peptides are designed for critical applications such as allergan-specific immunotherapy.

To summarize the foregoing results, if a given food allergan contains significant linear epitopes, which seems to be true for stable major allergans such as those of peanut and shrimp the SPOTs system may be more advantageous than the use of recombinant peptides libraries. However, if allergans are studied that contain more conformational epitopes, recombinant peptide libraries may help to identify the relevant epitopes.

Example 9

Immunotherapy

DNA sequences coding for modified and unmodified Pen a 1, Pen a 1 fragments, or Pen a 1 peptides will be administered subcutaneoudy, intramuscular, or orally. Dosage, frequency and duration of treatment will be adjusted on a individual basis. DNA immunization will employ naked DNA, plasmid DNA (pDNA) vectors encoding for modified and unmodified Pen a 1, Pen a 1 fragments, or Pen a 1 peptides as well as pDNA conjugated to immunostimulatory sequences (ISS). These ISS contain unmethylated cytosine and guanine dinucleotide repeat motifs. These CpG motifs and DNA molecules stimulate antigen-presenting cells and natural killer cells to secrete IFN-γ and IL-12, cytokines that promote immune deviation of T lymphocytes toward the T_H1 phenotype, away from the allergic T_H2 phenotype, and, thus reduce the production of allergen-specific IgE antibodies. These DNA molecules, plasmid DNA and ISS will be administered in several ways. They can be administered with DNA encoding the allergen (DNA immunization), given alone, or conjugated with modified and unmodified allergen, allergen fragments, and peptides.

Example 10

Antibodies sp cific for modified allerg ns

Modified and unmodified Pen a 1, Pen a 1 fragments, or Pen a 1 peptides will be used to produce specific antibodies in mice, rats, rabbits or other experimental animals. The produced sera and monoclonal antibodies will be used to detect, measure and standardize modified and unmodified Pen a 1, Pen a 1 fragments, or Pen a 1 peptides, that are produced for diagnostic and therapeutic purposes. In this context, monoclonal antibodies are especially useful as secondary standards, since monoclonal antibodies recognize only specific determinants (epitopes) of allergens and non-allergens; the specificity of an antibody depends on the uniqueness of the epitope. Furthermore, these antibodies and antisera will also be very useful to characterize cross-reacting epitopes on related and unrelated proteins.

Example 11

Transgenic crustacea

The DNA coding for the mutated tropomyosins will be developed by site-directed mutagenesis of Pen a 1. Using the spermatophore-microinjection (SMI) technique (Li SS, Tsai HJ Transfer of foreign gene to giant freshwater prawn (*Macrobrachium rosenbergii*) by spermatophore-microinjection (SMI). Mol. Reprod. Dev. (2000) Jun;56(2):149-154) the mutated Pen a 1 will introduced into the genome of shrimp or prawn species such as the giant freshwater prawn *Macrobrachium rosenbergii* or Tiger shrimp (*Penaeus monodon*). Approximately 1 µg of the circular plasmid DNA will be directly microinjected into spermatophores. Fertilization and hatching of shrimp or prawns created with SMI were completed in vivo. The genomes of free swimming, SMI-created larvae (21 days after fertilization) will be analyzed by PCR and Southern blot analyses.

It will be apparent that any of the modified tropomyosins disclosed herein including particular modified shrimp tropomyosins can be transduced into shrimp to make the transgenic shrimp. Such shrimp may or may not include tropomyosin "knock-out" mutations as needed for a particular application. Such transgenic shrimp will be especially useful in seafood farming applications featuring shrimp with reduced or negligible allergen potential.

The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated that, upon consideration of

the present specification and drawings, those skilled in the art may make modifications and improvements within the spirit and scope of this invention as defined by the claims.

The following references are cited throughout the specification. All documents mentioned herein, including the references listed below, are incorporated by reference.

1. FDA. Report of the FDA ad Hoc committee on hypersensitivity to food constituents. .S. Food and Drug Administration, Washington, D.C.1996.
2. Taylor SL, Bush RK. Allergy by ingestion of seafoods. In: ATT., ed. Marine Toxins and Venoms. New York: Marcel Dekker, Inc., 1988;3:149-183.
3. Leung PS, Chu KH, Chow WK, et al. J Allergy Clin Immunol 1994;94(5):882-890.
4. Shanti KN, Martin BM, Nagpal S, Metcalfe DD, Rao PV. J Immunol 1993;151(10):5354-5363.
5. Daul CB, Slattery M, Reese G, Lehrer SB. Int Arch Allergy Immunol 1994;105(1):49-55.
6. Mykles DL, Cotton JL, Taniguchi H, Sano K, Maeda Y. J Muscle Res Cell Motil 1998;19(2):105-115.
7. Leung PS, Chen YC, Mykles DL, Chow WK, Li CP, Chu KH. Molecular Marine Biology & Biotechnology 1998;7(1):12-20.
8. Leung PS, Chen YC, Gershwin ME, Wong SH, Kwan HS, Chu KH. J Allergy Clin Immunol 1998;102(5):847-852.
9. Miyazawa H, Fukamachi H, Inagaki Y, et al. J Allergy Clin Immunol 1996;98(5 Pt 1):948-953.
10. Ishikawa M, Ishida M, Shimakura K, Nagashima Y, Shiomi K.. Bioscience, Biotechnology & Biochemistry 1998;62(7):1337-1343.
11. Ishikawa M, Ishida M, Shimakura K, Nagashima Y, Shiomi K. J Food Science 1998;63:44-47.
12. Aki T, Kodama T, Fujikawa A, et al, J Allergy Clin Immunol 1995;96(1):74-83.
13. Asturias JA, Arilla MC, Gomez-Bayon N, Martinez A, Martinez J, Palacios R. Biochimica et Biophysica Acta 1998;1397(1):27-30.
14. Asturias JA, Gomez-Bayon N, Arilla MC, of et al. Journal of Immunology 1999;162(7):4342-4348.

15. Santos AB, Chapman MD, Aalberse RC, et al. J Allergy Clin Immunol 1999;104(2 Pt 1):329-337.

16. Martinez A, Martinez J, Palacios R, Panzani R. Allergol Immunopathol (Madr)1997;25(3):118-126.

17. van Ree R. Antonicelli L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi F. Allergy 1996;51(2):108-113.

18. Carrillo T, Rodriguez de Castro F, Blanco C, Castillo R, Quiralte J, Cuevas M. Ann Allergy 1994;73(6):504-508.

19. Banzet ML, Adessi B, Vuitton DA, L'Amecoforcal. Rev Fr Allergol 1992;32:198-202.

20. Reese G, Ayuso R, Lehrer SB. Int Arch Allergy Immunol 1999;119(4):247-258.

21. Smillie LB. Trends in Biochemical Sciences 1979;4:151-155.

22. Restani P, Fiocchi A, Beretta B, Velona T, Giovannini M, Galli CL. J Am Coll Nutr 1997;16(4):383-389.

23. Ayuso R, Lehrer SB, Lopez M, et al. Allergy 2000;55(4):348-354.

24. Lehrer SB, McCants ML. J Allergy Clin Immunol 1987;80(2):133-139.

25. Laemmli UK. Nature 1970;227(259):680-685.

26. Demeulemester CG, Peltre, G., Laurente, M. Electrophoresis 1987(8):71-73.

27. Kyhse-Andersen J. Electroblotting of multiple gels:. J Biochem Biophys Methods 1984;10(3-4):203-209.

28. Leary JJ, Brigati DJ, Ward DC. Proceedings of the National Academy of Sciences of the United States of America 1983;80(13):40454049.

29. Rabjohn P, Helm EM, Stanley JS, et al.. J Clin Invest 1999;103(4):535-542.

30. Stanley JS, King N, Burks AW, et al. Arch Biochem Biophys 1997;342(2):244-253.

31. Burks AW, Shin D, Cockrell G, Stanley JS, Helm RM, Bannon GA.. Eur J Biochem 1997;245(2):334-339.

32. Pomes A, Melen E, Vailes LD, Retief JD, Arruda LK, Chapman MD. JBiol Chem 1998;273(46):30801-30807.

33. Beezhold DH, Hickey VL, Slater JE, Sussman GL J Allergy Clin Immunol 1999;103(6):1166-1172.34. Muller U, Akdis CA, Fricker M, et al. J Allergy Clin Immunol 1998;101(6 Pt 1):747-754.

35. Leung PS, Chow WK, Duffey S, Kwan HS, Gershwin ME, Chu KH J Allergy Clin Immunol 1996;98(5 Pt 1):954-961.

36. Pascual CY, Crespo JF, San Martin S, et al. Allergy 1997;52(5):514520.

37. Reese G, Ayuso R, Carle T, Lehrer SB. International Archives of Allergy & Immunology 1999;11.8(2-4):300-301.

38. J.S. Stanley, N. King, A.W. Burks, S.K. Huang, H. Sampson, G. Cockrell, R.M. Helm, C.M. West, G.A. Bannon, Arch Biochem Biophys 342 (1997) 244.

39. A.W. Burks, N. King, G.A. Bannon, Int Arch Allergy Immunol 118 (1999) 313.

40. G.A. Bannon, D. Shin, S. Maleki, R. Kopper, A.W. Burks, Int Arch Allergy Immunol 118 (1999) 315.

What is claimed is:

1. A vaccine comprising at least one modified amino acid, of crustacea tropomyosin sequence; or a fragment thereof.

2. The vaccine of claim 1 wherein the modified amino acid sequence reduces binding between crustacea tropomyosin and an IgE antibody by at least 45% as determined by a standard IgE antibody test.

3. The vaccine of claim 2 wherein the modified amino acid sequences includes an epitope with at least one amino acid substitution that reduces the binding in the test.

4. The vaccine of claim 1 wherein the modified amino acid substitutions include at least one of a non-polar aliphatic group; a polar, uncharged group; an aromatic group; a positively charged group; or a negatively charged group.

5. A vaccine comprising crustacea tropomyosin or a fragment thereof.

6. A method for desensitizing an individual allergic to crustacea tropomyosin, the method comprising administering to the individual a desensitization sufficient amount of a vaccine comprising at least one modified amino acid of crustacea tropomyosin; or a vaccine comprising a modified fragment of crustacea tropomyosin.

7. The method according to claim 6 wherein the administration is by at least one of oral, suppository, parenteral, or subcutaneous administration.

8. A vector comprising nucleic acid sequence encoding a modified crustacea tropomyosin, the modification comprising at least one amino acid substitution or deletion in an epitope capable of binding an IgE antibody, wherein the vector can be selected from viruses, plasmids, bacterial, yeast.

9. The vector of claim 8 comprising modified gene fragments of crustacea tropomyosin.

10. The vector of claim 8 comprising at least one nucleic acid substitution which reduces binding between crustacea tropomyosin and an IgE antibody by at least 45% as determined by a standard IgE antibody test.

11. The vector of claim 8 or 9 wherein the nucleic acid substitutions code for modified peptides that abolish or reduce between crustacea tropomyosin and an IgE antibody by at least 45% as determined by a standard IgE antibody test.

12. A peptide comprising amino acid sequence of the tropomyosin molecule of crustacea, or any fragment thereof.

13. A peptide according to claim 12, wherein the crustacean tropomyosin is shrimp tropomyosin.

14. A peptide according to claim 12, wherein the peptide is five or more amino acids long.

15. A peptide according to claim 12, wherein the peptide overlaps by at least two amino acids with the next consecutive peptide at the 3' end of the previous peptide.

16. A peptide according to claim 12, wherein said peptide evokes a positive skin reaction in a patient sensitive to ingestion of crustaceans.

17. A method for desensitizing an individual allergic to crustacea. the method comprising administering to the individual a desensitization sufficient amount of the peptide of claim 12.

18. A peptide according to claim 12, comprising at least one amino acid substitution which reduces binding between crustacea tropomyosin and an IgE antibody by at least about 45% as determined by a standard IgE antibody test.

19. An amino acid substitution according to claim 18, wherein the substitutions can be selected from any group of amino acids which are different to the amino acid group that is being substituted.

20. An amino acid substitution according to claim 19, wherein the substituting amino acid include at least one of a non-polar aliphatic group; a polar, uncharged group; an aromatic group; a positively charged group; or a negatively charged group.

21. A peptide according to claim 18, wherein said crustacea tropomyosin-specific IgE antibodies are shrimp tropomyosin-specific IgE antibodies and said crustacea tropomyosin is shrimp tropomyosin.

22. A method for treating an individual allergic to crustacea, said method comprising administering to the individual a sufficient amount of the peptide of claim 18, which reduces binding between crustacea tropomyosin and an IgE antibody by at least about 45% as determined by a standard IgE antibody test.

23. The method according to claim 22, which can be used to perform allergan specific therapy of patients allergic to other crustaceans wherein the administration is by at least one of oral, suppository, parenteral, or subcutaneous administration.

24. A method according to claim 23, which reduces binding between crustacea tropomyosin and an IgE antibody by at least about 45% as determined by a standard IgE antibody test.

25. The method according to claim 23, which can be used to perform allergan specific therapy of patients allergic to arthropods.

26. A method according to claim 25, which reduces binding between arthropod tropomyosin and an IgE antibody by at least about 45% as determined by a standard IgE antibody test.

27. A vector comprising the unmodified gene of crustacea tropomyosin; or a fragment thereof, wherein the vector can be selected from viruses, plasmids, bacterial, yeast.

28. A method for desensitizing an individual allergic to crustacea tropomyosin, the method comprising administering to the individual a desensitization sufficient

amount of a vector comprising the unmodified gene of crustacea tropomyosin; or a vector comprising an unmodified fragment of crustacea tropomyosin.

29. The method according to claim 28 wherein the administration is by at least one of oral, suppository, parenteral, or subcutaneous administration.

30. A peptide comprising amino acid sequences of the five major allergenic regions of tropomyosin, and fragments thereof, wherein said peptides evoke a positive skin reaction in a patient sensitive to ingestion of crustaceans.

31. A peptide according to claim 30, wherein substitution of at least one amino acids reduces binding between crustacea tropomyosin and an IgE antibody by at least about 45% as determined by a standard IgE antibody test.

32. An amino acid substitution according to claim 30, wherein the substitutions can be selected from any group of amino adds which are different to the amino acid group that is being substituted.

33. An amino acid substitution according to claim 32, wherein the substituting amino acid include at least one of a non-polar aliphatic group; a polar, uncharged group; an aromatic group; a positively charged group; or a negatively charged group.

34. An amino acid substitution according to claim 30, wherein the substitution is located anywhere in the allergenic epitope.

35. An amino acid substitution according to claim 30, wherein the substitution is located in the center of the allergenic epitope.

36. A method for desensitizing an individual allergic to crustacea tropomyosin, the method comprising administering to the individual a desensitization sufficient amount of the peptide according to claim 30.

37. The method according to claim 36 wherein the administration is by at least one of oral, suppository, parenteral, or subcutaneous administration.

38. The method of claim 36 wherein the individual is allergic to shrimp tropomyosin.

39. The method of claim 36 wherein the individual is allergic to other crustacea tropomyosin.

40. The method of claim 36 wherein the individual is allergic to arthropod tropomyosin.

41. Use of any of the peptides in any one of the above claims and pharmaceutically acceptable derivatives and salts thereof for preparation of a medicament for the treatment of an inflammatory disorder.

42. Expression of any of the peptides in any one of the above claims, in recombinant host selected from the group comprising bacteria, yeast, fungi, insect, crustacea, and mammalian cells.

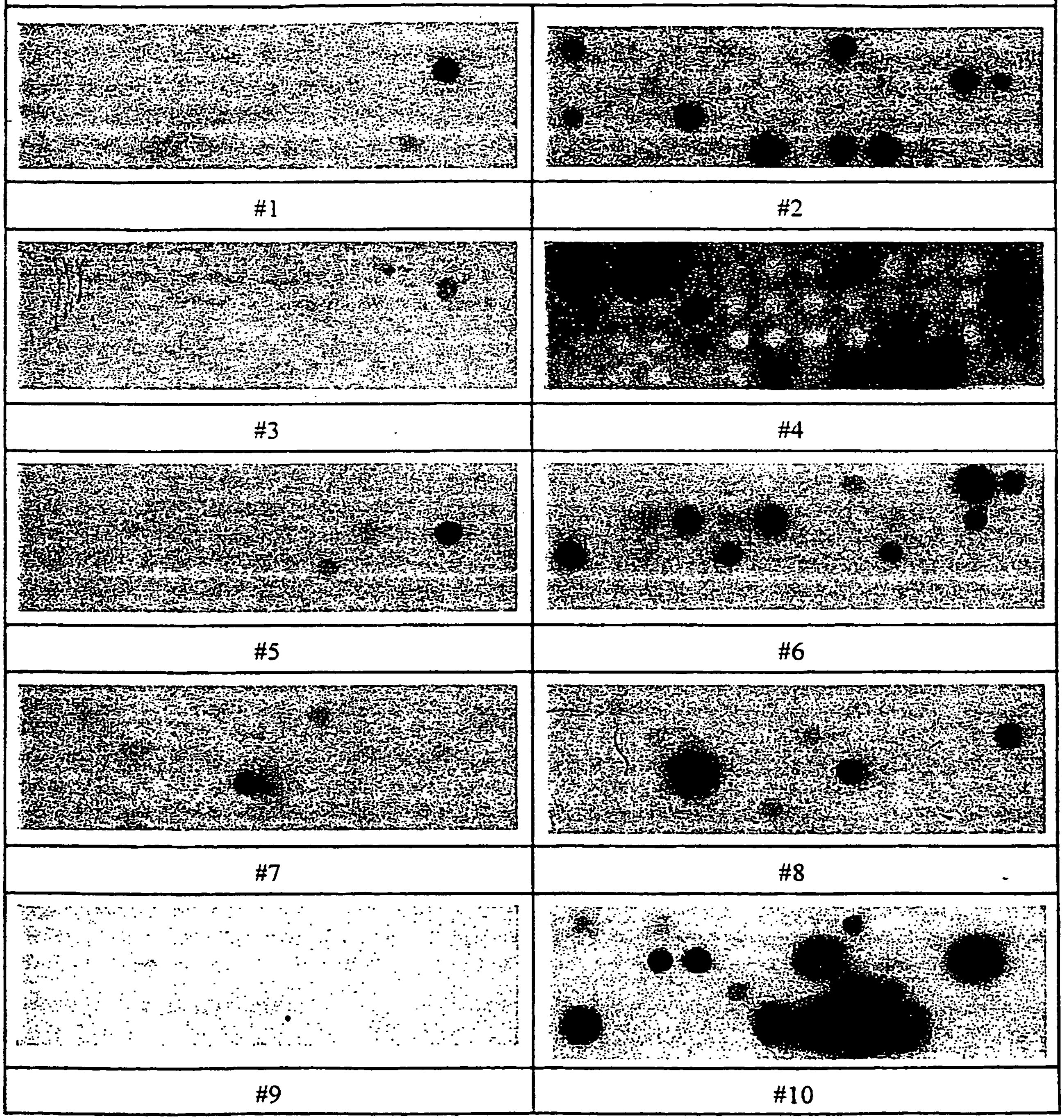
43. A transgenic animal expressing a modified allergan which is less reactive with IgE comprising at least one IgE binding site present in the allergan modified by at least one amino acid change so that the site no longer binds IgE.

44. An antibody that specifically binds a modified tropomysin molecule.

```
          1       10        20        30        40        50        60        70        80
Pen a 1   MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQVQESLLKANIQLVEKDKALSNA
1         MDAIKKKMQAMKLEK
2               KMQAMKLEKDNAMDR
3                     LEKDNAMDRADTLEQ
4                           MDRADTLEQQNKEAN
5                                 LEQQNKEANNRAEKS
6                                       EANNRAEKSEEEVHN
7                                             EKSEEEVHNLQKRMQ
8                                                   VHNLQKRMQQLENDL
9                                                         RMQQLENDLDQVQES
10                                                              NDLDQVQESLLKANI
11                                                                    QESLLKANIQLVEKD
12                                                                          ANIQLVEKDKALSNA

                80        90        100       110       120       130       140       150
Pen a 1            EGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAADESERMRKVLENRSLSDEERMDALENQLKEARF
13        EKDKALSNAEGEVAA
14              SNAEGEVAALNRRIQ
15                    VAALNRRIQLLEEDL
16                          RIQLLEEDLERSEER
17                                EDLERSEERLNTATT
18                                      EERLNTATTKLAEAS
19                                            ATTKLAEASQAADES
20                                                  EASQAADESERMRKV
21                                                        DESERMRKVLENRSL
22                                                              RKVLENRSLSDEERM
23                                                                    RSLSDEERMDALENQ
24                                                                          ERMDALENQLKEARF

                150       160       170       180       190       200       210       220
Pen a 1            LAEEADRKYDEVARKLAMVEADLERAEERAETGESKIVELEEELRVVGNNLKSLEVSEEKANQREEAYKEQI
25        ENQLKEARFLAEEAD
26              ARFLAEEADRKYDEV
27                    EADRKYDEVARKLAM
28                          DEVARKLAMVEADLE
29                                LAMVEADLERAEERA
30                                      DLERAEERAETGESK
31                                            ERAETGESKIVELEE
32                                                  ESKIVELEEELRVVG
33                                                        LEEELRVVGNNLKSL
34                                                              VVGNNLKSLEVSEEK
35                                                                    KSLEVSEEKANQREE
36                                                                          EEKANQREEAYKEQI

                220       230       240       250       260       270       280 284
Pen a 1            KTLTNKLKAAEARAEFAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSELSGY
37        REEAYKEQIKTLTNK
38              EQIKTLTNKLKAAEA
39                    TNKLKAAEARAEFAE
40                          AEARAEFAERSVQKL
41                                FAERSVQKLQKEVDR
42                                      QKLQKEVDRLEDELV
43                                            VDRLEDELVNEKEKY
44                                                  ELVNEKEKYKSITDE
45                                                        EKYKSITDELDQTFS
46                                                              ITDELDQTFSELSGY
```

Figure 1: Forty-six, synthetic overlapping peptides spanning the entire sequence of Pen a 1 (length: 15 amino acid residues, offset: 6 amino acids)

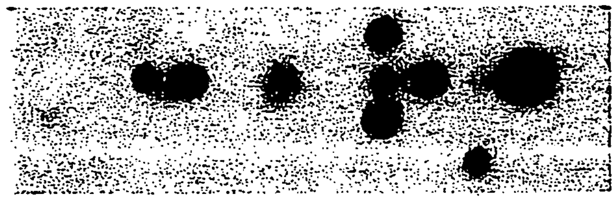
Figure 2
PepScan Analysis PepScan analysis of Pen a 1 peptides with
sera from 18 shrimp-allergic subjects.
(peptide length: 15 aa, offset: 6 aa)



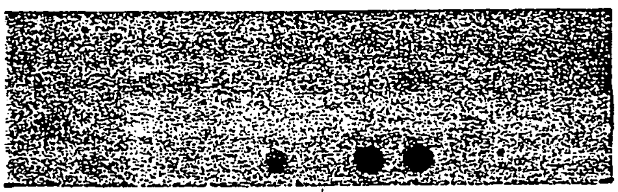
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10

Figure 2 (continued)
PepScan Analysis PepScan analysis of Pen a 1 peptides with
sera from 18 shrimp-allergic subjects.
(peptide length: 15 aa, offset: 6 aa)

#11

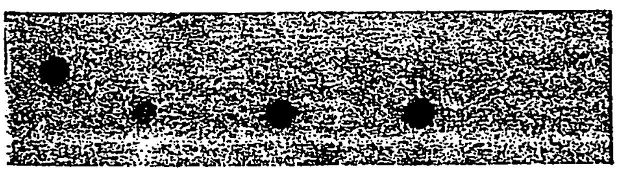


#12

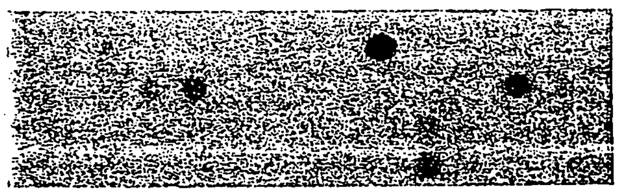


#13

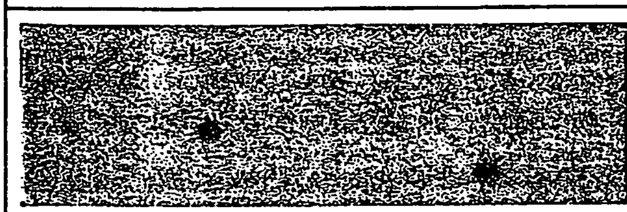
#14



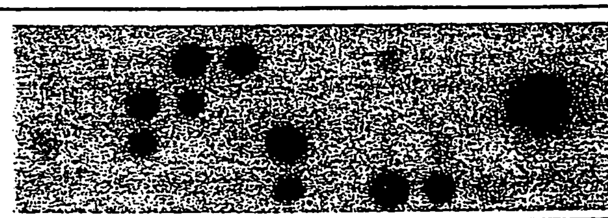
#15



#16



#17



#18

Figure 3.1:
Region 1: individually recognized epitopes and sequence comparison with allergenic and non-allergenic tropomyosins

	Position	Sequence	Subject	IgE-reactive peptides during initial screening
		37 40 50 60 63		
Pen a 1	position	EKSEEEVHNLQKRMQQLENDLDQVQES		
peptide used for initial screening	43-57	VHNLQKRMQQLENDL	peptide 8	
	43-55	VHNLQKRMQQLEN	2	8
	43-55	VHNLQKRMQQLEN	4	8
	43-55	VHNLQKRMQQLEN	12	8
	43-55	VHNLQKRMQQLEN	16	8
Met e 1		....		
Hom a 1		....		
HomaTMs		..T...IRITH.K...V..E......Q		
Pan s 1		..A........................		
Per a 7		..A...ARS...KI..I......TM.Q		
Der p 10		.......RA...KI..I..E......Q		
Der f 10		.......RA...KI..I..E......Q		
GalgαTM		KQL.D.LVA...KLKGT.DE..KYS..		
GalgβTM		KQL...QQG...KLKGT.DEVEKYS..		
SussαTM1		KQL.D.LVS...KLKAT.DE..KYS.A		
OrycβTM2		KQL...QQA...KLKGT.DEVEKYS..		
SaltTM1		KQHDDALIQM..KLKGT.DE..KYS.A		
SaltTM2		KQL.D.LLS...NLKGT.DE..KYS.A		

Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Metapenaeus ensis* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMs), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Insecta: *Periplaneta america* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus*, *D. farinae* (house dust mites, Der p 10, Der f 10). Vertebrata: *Gallus Gallus* (chicken, alpha-tropomyosin GalgαTM; beta-tropomyosin GalgβTM), *Sus scrofa* (pig, SussβTM), *Salmo trutta* (Atlantic salmon, SaltTM), *Oryctolagus cuniculus* (rabbit, OrycβTM) (GenBank data).

Figure 3.2:

Region 2: individually recognized epitopes and sequence comparison with allergenic and non-allergenic tropomyosins

```
                                    70          80          90          100         110         120
                                    |           |           |           |           |           |
Pen a 1                  position   QLVEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA
peptides used for        85-99                        VAALNRRIQLLEEDL                          peptide 15
initial screening        91-105                             RIQLLEEDLERSEER                    peptide 16
                         96-111                                   EDLERSEERLNTATT              peptide 17
```

	Position		Subject	IgE-reactive peptides during initial screening
	87-101	ALNRRIQLLEEDLER	6	15, 16, 17
	87-101	ALNRRIQLLEEDLER	10	15, 16
	87-101	ALNRRIQLLEEDLER	18	15, 16
	91-101	RIQLLEEDLER	12	15, 16

```
Met e 1      ...................................................
Hom a 1      ..E................................................
HomaTMs      K.E..E...Q.........................................
Pan s 1      ..E................................................
Per a 7      K.D......Q...S.........................A...A........V
Der p 10     K.E..E...QT...D.........I..........KI..A..E....S.
GalgαTM      K.ELA..KATD..S...S.......V..E.D.AQ...A..LQ..E..EK..
GalgβTM      K.ELA..KATD..S...S.......V..E.D.AQ...A..LQ..E..EK..
SussαTM1     K.ELA..KATD..S...S.......V..E.D.AQ...A..LQ..E..EK..
OrycαTM1     K.ELA..KATD..S...S.......V..E.D.AQ...A..LQ..E..EK..
OrycβTM2     K.ELA..KATD..S...S.......V..E.D.AQ...A..LQ..E..EK..
SaltTM1      K.ELA..KATD..S...S.......V..E.D.AQ...A..LQ..E..EK..
SaltTM2      K.ELA..KATD..S...S.......V..E.D.AQ...A..LQ..E..EK..
```

Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Metapenaeus ensis* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMs), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Insecta: *Periplaneta america* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus*, *D. farinae* (house dust mites, Der p 10, Der f 10). V rtebrata: *Gallus Gallus* (chicken, alpha-tropomyosin GalgαTM; beta-tropomyosin GalgβTM), *Sus scrofa* (pig, SussβTM), *Salmo trutta* (Atlantic salmon, SaltTM), *Oryctolagus cuniculus* (rabbit, OrycβTM) (GenBank data).

Figure 3.3:
Region 3: individually recognized epitopes and sequence comparison with allergenic and non-allergenic tropomyosins

		125 130 140 150 160	
Pen a 1, Hom a 1, Pan s 1	position	RMRKVLENRSLSDEERMDALENQLKEARFLAEEADRK	
peptides used for	133-143	RSLSDEERMDALENQ	Peptide 23
initial screening	145-159	ERMDALENQLKEARF	Peptide 24

Position		Subject	IgE-reactive peptides during initial screening
137-145	DEERMDALE	18	23
137-145	DEERMDALE	10	23
133-141	RSLSDEERM	12	23
135-146	LSDEERMDALEN	1	23
134-141	SLSDEERM	2	23
136-143	SDEERMDA	5	23
144-151	LENQLKEA	6	23
139-153	ERMDALENQLKEARF	4	24

Met e 1	..................[illegible]..........
Hom a 1	..................[illegible]..........
HomaTMs	..................[illegible]..........
Pan s 1	..................[illegible]..........
Per a 7	.A..I..SKG.A......[illegible]..M.....K.
Der p 10	....M..H..IT.....EG[illegible].MM..D....
Der f 10	....M..H..IT......G[illegible].MM..D....
GalgαTM	.GM..I...AQK...K.EIQ[illegible]KHI.......
GalgβTM	.GM..I...AMK...K.ELQ[illegible]KHI.......
SussαTM1	.GM..I.S.AQK...K.EIQ[illegible]KHI..D....
OrycαTM1	.GM..I.S.AQK...K.EIQ[illegible]KHI..D....
OrycβTM2	.GM..I...AMK...K.ELQ[illegible]KHI..DS...
SaltTM1	.GM..I...ASK...K.EMQ[illegible]KHI.......
SaltTM2	.GM..I...ASK...K.EIQ[illegible]KHI.......

Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Metapenaeus ensis* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMs), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Insecta: *Periplaneta america* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus*, *D. farinae* (house dust mites, Der p 10, Der f 10). Vertebrata: *Gallus Gallus* (chicken, alpha-tropomyosin GalgαTM; beta-tropomyosin GalgβTM), *Sus scrofa* (pig, SussβTM), *Salmo trutta* (Atlantic salmon, SaltTM), *Oryctolagus cuniculus* (rabbit, OrycβTM) (GenBank data).

Figure 3.4:
Region 4: individually recognized epitopes and sequence comparison with allergenic and non-allergenic tropomyosins

```
                                      180         190         200        209
                                       |           |           |          |
Pen a 1, Hom a 1, Pan s 1  position  AEERAETGESKIVELEEELRVVGNNLKSLEV
peptides used for          187-201             ESKIVELEEELRVVG                 Peptide 32
initial screening
---------------------------------------------------------------------------------------------------
                                                                                          IgE-reactive peptides
                           Position                                            Subject    during initial screening
                           187-201             ESKIVELEEELRVVG                 #8         32
                           187-199             ESKIVELEEELRV                   #12        32
                           187-197             ESKIVELEEEL                     #10        32
---------------------------------------------------------------------------------------------------
Metel                                 ...............................
HomaTMf                               ...............................
HomaTMs                               ...............................
Pans1                                 ...........F...................
Pera7                                 ......S........................
Derp10                                ...............................
Derf10                                ...............................
GalgαTM                               ......LS...CA......KT.T.......A
GalgβTM                               S.....VA...CGD.....KI.T.......A
SussαTM1                              ......LS.G.CA......KT.T.......A
OrycαTM1                              ......LS.G.CA......KT.T.......A
OrycβTM1                              S.....VA...CGD.....KI.T.......A
SaltTM1                               T.....LA.G.CA......KN.S.......A
SaltTM2                               ......VS.L.CSD.....KN.T.......A
```

Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Metapenaeus ensis* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMs), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Insecta: *Periplan ta america* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus*, *D. farinae* (house dust mites, Der p 10, Der f 10). Vertebrata: *Gallus Gallus* (chicken, alpha-tropomyosin GalgαTM; beta-tropomyosin GalgβTM), *Sus scrofa* (pig, SussβTM), *Salmo trutta* (Atlantic salmon, SaltTM), *Oryctolagus cuniculus* (rabbit, OrycβTM) (GenBank data).

Figure 3.5:

Region 5: individually recognized epitopes and sequence comparison with allergenic and non-allergenic tropomyosins

```
                                          239      250      260      270       280 284
                                           |        |        |        |         |  |
Pen a 1, Hom a 1, Pan s 1                  AEFAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSELSGY
                              247-261              QKLQKEVDRLEDELV                    peptide 42
peptides used for             253-267                    VDRLEDELVNEKEKY              peptide 43
initial screening             259-273                         ELVNEKEKYKSITDE         peptide 44
                              265-279                               EKYKSITDELDQTFS   peptide 45
                              270-284                                    ITDELDQTFSELSGY  peptide 46
```

	Position		Subject	IgE-reactive peptides during initial screening
	249-260	LQKEVDRLEDEL	2	42, 44, 45
	249-261	LQKEVDRLEDELV	10	42, 43, 44, 45
	251-259	KEVDRLEDE	4	42, 44, 45, 46
	266-273	KYKSITDE	2	42, 44, 45
individual epitopes	266-273	KYKSITDE	10	42, 43, 44, 45
	266-273	KYKSITDE	4	42, 44, 45, 46
	266-273	KYKSITDE	18	42, 44, 45, 46
	273-281	ELDQTFSEL	4	42, 44, 45, 46

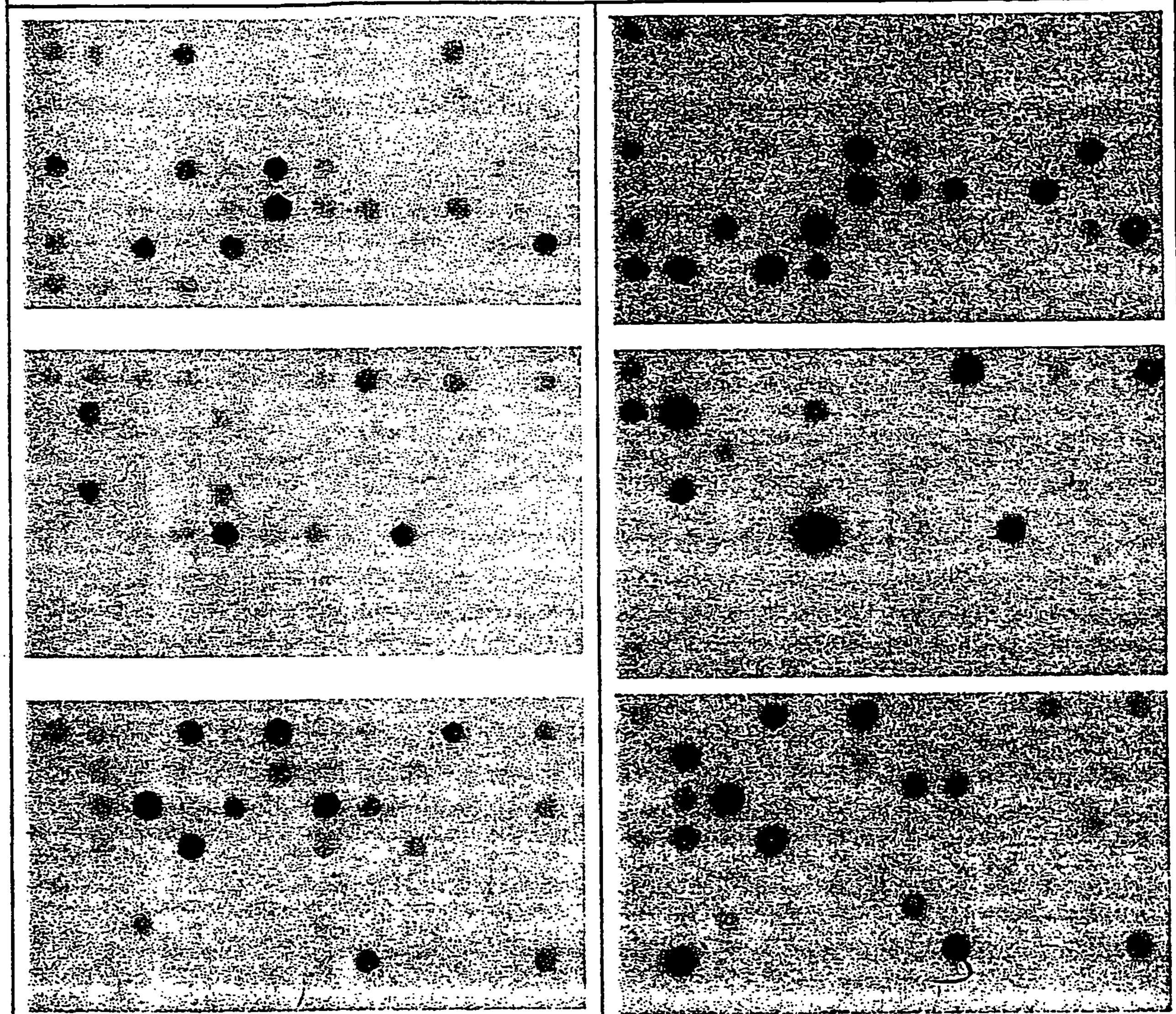
```
Met e 1     ..........................................
Homa 1      ..........................................
HomaTMs     ..........................................
Pan s 1     ..........................................
Per a 7     ..................H......F.C..M..T..A..
Der p 10    ..................H........S......A..T..
Der f 10    ..................H........S......A..T..
GalgαTM     ........T..E.SI.D....YAQ.L...A.SE..HALNDMTSI
GalgβTM     ........A..E.TI.D....VYAQ.M...A.SE..NALNDITSL
SussαTM1    ........T..E.SI.D....YAQ.L...A.SE..HALNDMTSI
OrycαTM     ........T..E.SI.D....YAQ.L...A.SE..HALNDMTSI
OrycβTM2    ........A..E.TI.D....VYAQ.M...A.SE..NALNDITSL
SaltTM1     ........A..E.TI.D....YAQ.L...A.SE..HALNDMTSI
SaltTM2     ......T.A..E.SI.D....YAQ.L...A.SE..HALNDMTSI
```

Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Metapenaeus ensis* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMs), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Insecta: *P riplaneta america* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus*, *D. farinae* (house dust mites, Der p 10, Der f 10). Vertebrata: *Gallus Gallus* (chicken, alpha-tropomyosin GalgαTM; beta-tropomyosin GalgβTM), *Sus scrofa* (pig, SussβTM), *Salmo trutta* (Atlantic salmon, SaltTM), *Oryctolagus cuniculus* (rabbit, OrycβTM) (GenBank data).

```
Pen a 1      ALSNAEGE
# 2          K.......
# 3          .A......
# 4          ..T.....   1 substitution
# 5          ...D....
# 6          ......S.
# 7          KA......
# 8          K.T.....
# 9          K..D....
# 10         K.....S.
# 11         .AT.....
# 12         .A.D....   2 substitutions
# 13         .A....S.
# 14         ..TD....
# 15         ..T...S.
# 16         ...D..S.
# 17         KAT.....
# 19         KA.D....
# 19         KA....S.
# 20         K.TD....
# 21         K.T...S.
# 22         K..D..S.   3 substitutions
# 23         .ATD....
# 24         .AT...S.
# 25         .A.D..S.
# 26         ..TD..S.
# 27         KATD....
# 28         KAT...S.
# 29         KA.D..S.   4 substitutions
# 30         K.TD..S.
# 31         .ATD..S.
chicken Tm   KATD..S.   - 5 substitutions
```

Figure 4: Peptides, resulting from combinatorial substitutions and transforming a Pen a 1 peptide into the homologous chicken tropomyosin (Tm) sequence.

Figure 5.1
Combinatorial Substitutions
Epitopes of region 1



#12	#4

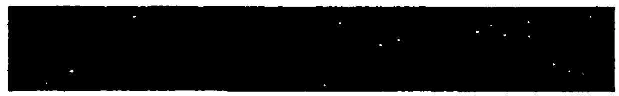
Figure 5.2 Combinatorial Substitutions Region 2	
	
#6	#10
not done	
#18	# 12

Figure 5.3
Combinatorial Substitutions
Epitopes of region 3

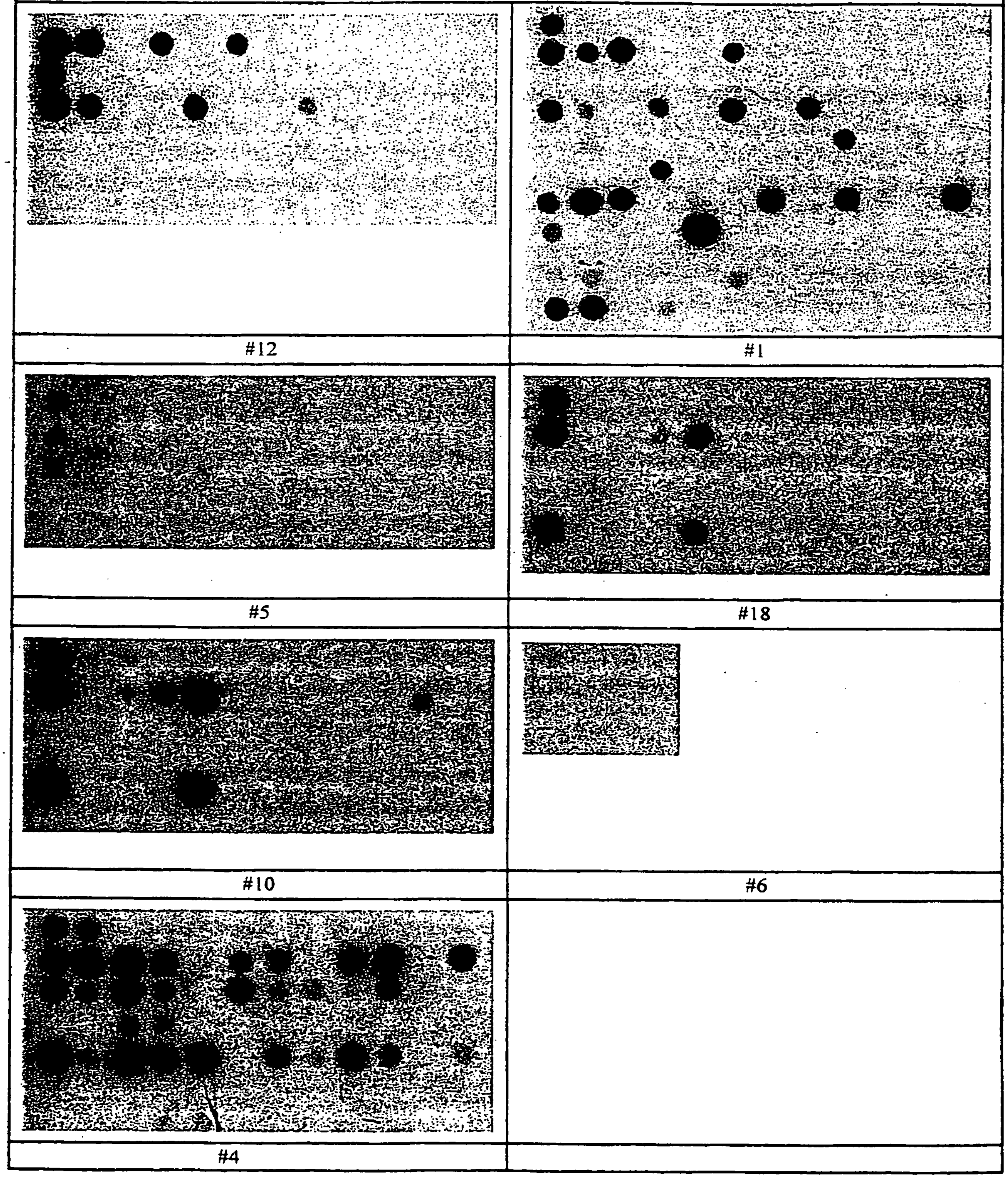
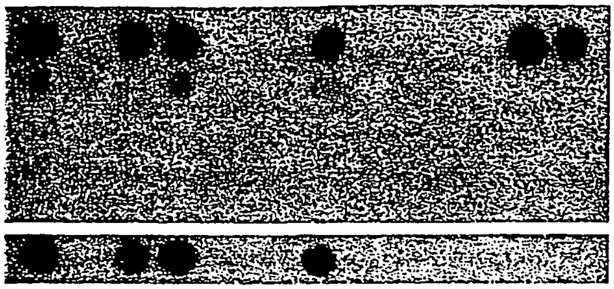
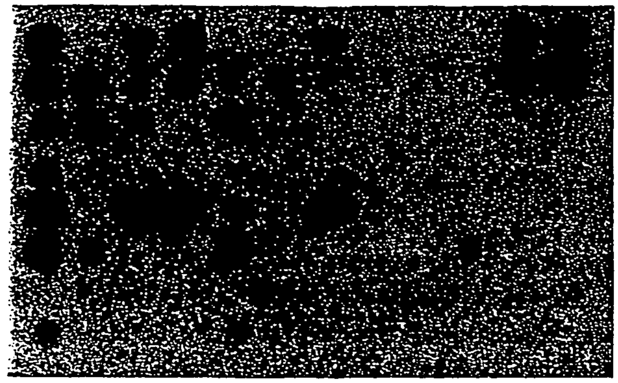


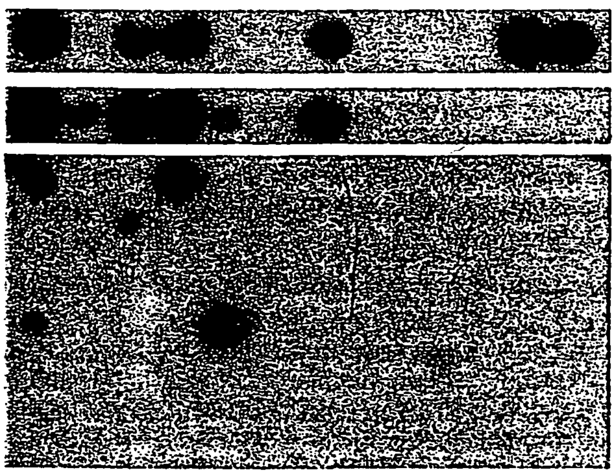
Figure 5.4
Combinatorial Substitutions
Epitopes of region 5



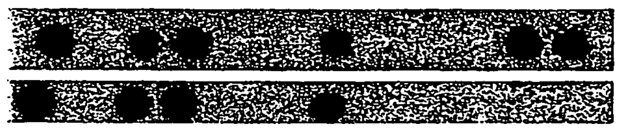
#6



#4



#10



#18

Figure 6

Critical Amino Acid Positions within Epitopes of Region 1

Pen a 1

37 40 50 60 63

position	EKSEEEVHNLQKRMQQLENDLDQVQES	critical position	mutated into amino acid	Subject
43-55	VHNLQKRMQQLEN	no	--	4
43-55	VHNLQKRMQQLEN	no	--	12

Critical Amino Acid Positions within Epitopes of Region 2

Pen a 1

70 80 90 100 110 120

position	QLVEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA	critical position	mutated into amino acid	Subject
87-101	ALNRRIQLLEEDLER	95	V, F	6
87-101	ALNRRIQLLEEDLER	98	E	6
87-101	ALNRRIQLLEEDLER	100	D	6
87-101	ALNRRIQLLEEDLER	95	V, F	10
91-101	RIQLLEEDLER	95	F	12

Critical Amino Acid Positions within Epitopes of Region 3

Pen a 1

125 130 140 150 160

position	RMRKVLENRSLSDEERMDALENQLKEARFLAEEADRK	critical position	mutated into amino acid	Subject
137-145	DEERMDALE	140	K	18
137-145	DEERMDALE	142	E	18
137-145	DEERMDALE	140	K	10
137-145	DEERMDALE	142	E	10
133-141	RSLSDEERM	135	Q, M, S	12
133-141	RSLSDEERM	136	K	12
135-146	LSDEERMDALEN	136	K	1
135-146	LSDEERMDALEN	142	E	1
136-143	SDEERMDA	136	K	5
136-143	SDEERMDA	140	K	5
136-143	SDEERMDA	142	E	5
144-151	LENQLKEA	144	Q	6
139-153	ERMDALENQLKEARF	144	Q	4

Critical Amino Acid Positions within Epitopes of Region 5

Pen a 1

239 250 260 270 280 284

position	AEEAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSELSGY	critical position	mutated into amino acid	Subject
249-260	LQKEVDRLEDEL	255	D	2
249-261	LQKEVDRLEDELV	250	E	10
249-261	LQKEVDRLEDELV	252	S	10
249-261	LQKEVDRLEDELV	255	D	10
249-261	LQKEVDRLEDELV	260	V	10
251-259	KEVDRLEDE	255	D	4
266-273	KYKSITDE	269	F	2
266-273	KYKSITDE	269	F	10
266-273	KYKSITDE	269	F	4
266-273	KYKSITDE	269	F	18
273-281	ELDQTFSEL	277	A	4
273-281	ELDQTFSEL	278	L	4
273-281	ELDQTFSEL	280	Q	4
273-281	ELDQTFSEL	281	M, I	4

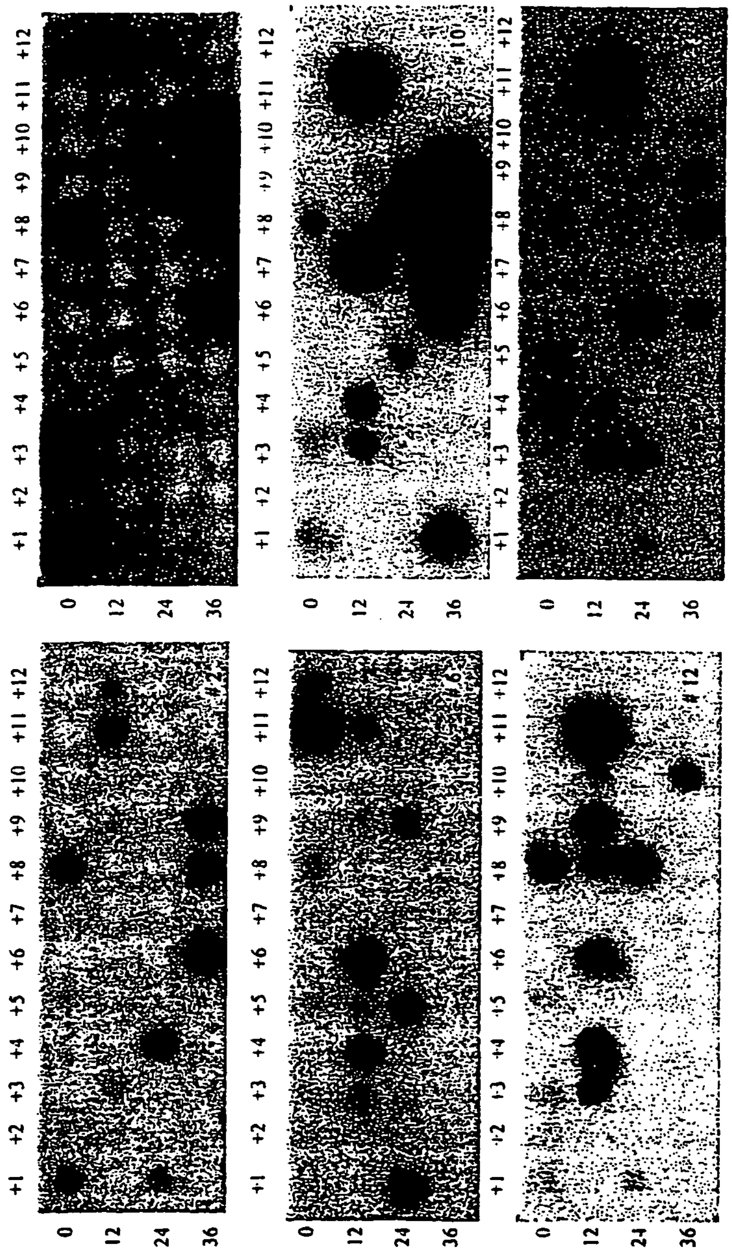


Figure 7

figure 7

```
            1    10   20   30   40   50   60   70   80   90   100

Pen a 1   MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQVQESLLKANIQLVEKDKALSNAEGEVAALNRRIQLLEEDLE
Pen i 1              ......A..E..RMLTU.DE..VT...........................................
Met e 1   ....................................................................N..................................
HomaTMf          .................................I..........................T..E..............................
HomaTMs          .................................I...T...IRITH.K...V..E......Q.SL..TK.E..E...Q....................
Pan s 1   ................................I....A...........................T..E..........................
Per a 7   ....................C.LLC...ARD..L....A...ARS...KI..I......TM.Q.MQV.AK.D......Q...S.................
Der f 10 .E.................I...EIA..KARD..L..........RA...KI..I..E......Q.SA..TK.E..E...QT...D..........I.....
Der p 10         .E.................I...EIA..KARD..L.........RA...KI..I..E.....Q.SA..TK.E..E...QT...D..........I.....
Tur c 1                                AAN....F.N.N.Q.QD.LSK                .T.......
Cra g 1          E..Q...EQ....LRDTEEQKA.I..DLTS... HSN...NF.T.N.K          TA.E..Q.IQS...........M.

            101  110  120  130  140  150  160  170  180  190  200

Pen a 1   RSEERLNTATTKLAEASQAADESERMRKVLENRSLSDEERMDALENQLKEARFLAEEADRKYDEVARKLAMVEADLERAEERAETGESKIVELEEELRVV
Pen i 1   .       .............        ...............       ....Q..............
Met e 1   ...........................................................................................
HomaTMf          ..............................................................................................
HomaTMs          ..............................................................................................
Pan s 1   ....................................................................................F.........
Per a 7   ......A...A........V.....A..I..SKG.A.................M......K.........................S...............
Der f 10  ......KI..A..E.....S.........M..H..IT......G.........MM..D.........................................
Der p 10         ......KI..A..E....S.........M..H..IT.....EG.........MM..D..........................................
Tur c 1   .N....Q...ER.E...K                            YI..D.E..      ..IT..V......A.L.AA.A.
Cra g 1   ......Q...E.            ....LNNAS...T.V..K     LI.....K      ..IT..V......A.L.AA.A.VY....Q.S..

            201  210  220  230  240  250  260  270  280 284

Pen a 1   GNNLKSLEVSEEKANQREEAYKEQIKTLTNKLKAAEARAEFAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSELSGY
Pen i 1   .....       .K...E..N...        ......        ...........Q
Met e 1   .......................................................................................
HomaTMf          ...........................A..........................................................
HomaTMs          ...........................A..........................................................
Pan s 1   ...........................................................................................
Per a 7   ...............L...E..Q......TR..E..........................H.......F.C.D..M..T..A..
Der f 10  ...............Q.......EQ..RIM.A...E..........................H.........S........A..T..
Der p 10         ...............Q.....HEQ..RIM.T...E..........................H.........S........A..T..
Tur c 1   ....I..QE.S...DS.E.T.RD..QR..          T.S.............LA.......A.S........A..A..
Cra g 1   A..I.T.Q.QNDQ.S...DS.E.T.RD..QRT.D..N..TE...T.S.   .........LA..   A.S.......A..N.
```

Figure 8: Comparison of identified Pen a 1 IgE-binding epitopes with homologous sequences of arthropod tropomyosins from the shrimp Metapenaeus ensis (Met e 1), American lobster Homarus americanus slow and fast isoforms (Hom a Tms, Hom a Tmf), spiny lobster Panulirus stimpsoni (Pan s 1), house dust mites Dermatophagoides pteronissinus and D. farinae (Der p 10, Der f 10) and cockroach Periplaneta americana (Per a 7), snail Turbo cornutus, and oyster Crassostrea gigas.

Figure 8

IgE-binding, recombinant peptides		non-IgE-binding synthetic peptides	
157-169	EADRKYDEVARKL	157 - 171	EADRKYDEVARKLAM
167-179	RKLAMVEADLERA	163 - 177	DEVARKLAMVEADLE
		169 - 183	LAMVEADLERAEERA

Figure 9: Sequence comparison of IgE-binding, recombinant peptides and non-IgE-binding synthetic peptides: Identical sequences are shaded.

Figure 9

```
             1        10        20        30        40        50        60        70        80        90       100
Pen a 1      MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQVQESLLKANIQLVEKDKALSNAEGEVAALNRRIQLLEEDLE
variety 1    .E.......ML.......L.A..QA.ADK.A.E.........LIQN..KLKGT.DE.............VV..S....................V..E.D
variety 2    .E.......ML.......L.A..QA.ADK.A.E.........LIQN..KLKGT.DE.............VV..S....................F..E.D
variety 3    .E.......ML.......L.A..QA.ADK.A.E.........LIQM..KLKGT.DE.............VV..S....................V..E.D
variety 4    .E.......ML.......L.A..QA.ADK.A.E.........LIQM..KLKGT.DE.............VV..S....................F..E.D
variety 5    .E.......ML.......L.A..QA.ADK.A.E.........LIQM..KLKGT.DE.............VV..S....................V..E.D
variety 6    .E.......ML.......L.A..QA.ADK.A.E.........LIQM..KLKGT.DE.............VV..S....................F..E.D
variety 7    .E.......ML.......L.A..QA.ADK.A.E.........LIQM..KLKGT.DE.............VV..S....................V..E.D
variety 8    .E.......ML.......L.A..QA.ADK.A.E.........LIQM..KLKGT.DE.............VV..S....................F..E.D
variety 9    .E.......ML.......L.A..QA.ADK.A.E.........LIQM..KLKGT.DE.............VV..S....................V..E.D
variety 10   .E.......ML.......L.A..QA.ADK.A.E.........LIQM..KLKGT.DE.............VV..S....................F..E.D
variety 11   .E.......ML.......L.A..QA.ADK.A.E.........LIQM..KLKGT.DE.............VV..S....................V..E.D
variety 12   .E.......ML.......L.A..QA.ADK.A.E.........LIQM..KLKGT.DE.............VV..S....................F..E.D

             101      110       120       130       140       150       160       170       180       190       200
Pen a 1      RSEERLNTATTKLAEASQAADESERMRKVLENRSLSDEERMDALENQLKEARFLAEEADRKYDEVARKLAMVEADLERAEERAETGESKIVELEEELRVV
variety 1    ..Q...A..LQ..E..E........GM..I...AQK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.
variety 2    ..Q...A..LQ..E..E........GM..I...AQK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.
variety 3    ..Q...A..LQ..E..E........GM..I...AMK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.
variety 4    ..Q...A..LQ..E..E........GM..I...AMK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.
variety 5    ..Q...A..LQ..E..E........GM..I...ASK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.
variety 6    ..Q...A..LQ..E..E........GM..I...ASK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.
variety 7    ..Q...A..LQ..E..E........GM..I...AQK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.
variety 8    ..Q...A..LQ..E..E........GM..I...AQK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.
variety 9    ..Q...A..LQ..E..E........GM..I...AMK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.
variety 10   ..Q...A..LQ..E..E........GM..I...AMK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.
variety 11   ..Q...A..LQ..E..E........GM..I...ASK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.
variety 12   ..Q...A..LQ..E..E........GM..I...ASK...K.E.Q.I.....KHI.......E......VII.S....S.....IS.G.C.D.....KT.

             201      210       220       230       240       250       260       270       280 284
Pen a 1      GNNLKSLEVSEEKANQREEAYKEQIKTLTNKLKAAEARAEFAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSELSGY
variety 1    T.................K.E.E..V..D..................E.S..D...V......F........AL.QN...
variety 2    T.................K.E.E..V..D..................E.S..D...V......F........AL.QM...
variety 3    T.................K.E.E..V..D..................E.S..D...V......F........AL.QM...
variety 4    T.................K.E.E..V..D..................E.S..D...V......F........AL.QM...
variety 5    T.................K.E.E..V..D..................E.S..D...V......F........AL.QM...
variety 6    T.................K.E.E..V..D..................E.S..D...V......F........AL.QM...
variety 7    T.................K.E.E..V..D..................E.S..D...V......F........AL.QI...
variety 8    T.................K.E.E..V..D..................E.S..D...V......F........AL.QI...
variety 9    T.................K.E.E..V..D..................E.S..D...V......F........AL.QI...
variety 10   T.................K.E.E..V..D..................E.S..D...V......F........AL.QI...
variety 11   T.................K.E.E..V..D..................E.S..D...V......F........AL.QI...
variety 12   T.................K.E.E..V..D..................E.S..D...V......F........AL.QI...
```

Figure 10: This figure shows 12 Pen a 1 varieties that contain substitutions in 78 positions and will reduce or abolish the IgE antibody reactivity of the Pen a 1 molecule ~~(see Table 8)~~. These varieties represent sequences that contain the maximal number of substitutions; not all substitutions may be necessary since the combined effect of multiple substitutions is not always known.

Figure 10

subjects	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	
# 1																																															2
# 2																																															10
# 3																																															1
# 4																																															15
# 5																																															6
# 6																																															13
# 7																																															9
# 8																																															6
# 9																																															1
# 10																																															15
# 11																																															5
# 12																																															14
# 13																																															7
# 14																																															2
# 15																																															8
# 16																																															8
# 17																																															6
# 18																																															16
# of positive subjects (+)	5	6	4	2	3	-	-	10	-	-	1	3	2	-	13	9	1	2	2	1	8	1	10	4	7	-	5	4	2	3	-	5	6	-	-	1	1	1	-	-	-	6	2	5	7	6	
intensity (i)	7	6	6	4	5	-	-	18	-	-	3	5	3	-	20	18	1	5	4	2	10	1	20	9	9	-	9	8	3	8	-	12	10	-	-	1	3	1	-	-	-	14	4	15	18	10	
≥ 50% of subjects								■							■	■							■																								
> mean intensity + 1 SDEV								■							■	■							■									■										■		■	■		

reactivity score: □ low (1) medium (2) ■ high (3)

Tabl 1: Summary of PepScan analysis of IgE antibody reactivities of 18 shrimp-allergic subjects to 46 overlapping peptides spanning the entire length of Pen a 1.

Table 2

IgE-reactive, peptides identified by PepScan of Pen a 1		IgE-reactive, peptides identified by SPOTSIZE of Pen a 1 region 1		IgE-reactive, peptides identified by SPOTSIZE of Pen a 1 region 2	
Pen a 1 1-15	MDAIKKKMQAMKLEK	Pen a 1 43-57	VHNLQKRMQQLENDL	Pen a 1 85-99	VAALNRRIQLLEEDL
Pen a 1 7-21	KMQAMKLEKDNAMDR	Pen a 1 43-56	VHNLQKRMQQLEND	Pen a 1 86-100	AALNRRIQLLEEDLE
Pen a 1 13-27	LEKDNAMDRADTLEQ	Pen a 1 43-55	VHNLQKRMQQLEN	Pen a 1 87-101	ALNRRIQLLEEDLER
Pen a 1 19-33	MDRADTLEQQNKEAN	Pen a 1 43-54	VHNLQKRMQQLE	Pen a 1 88-101	LNRRIQLLEEDLER
Pen a 1 25-39	LEQQNKEANNRAEKS	Pen a 1 44-56	HNLQKRMQQLEND	Pen a 1 88-102	LNRRIQLLEEDLERS
Pen a 1 43-57	VHNLQKRMQQLENDL	Pen a 1 45-57	NLQKRMQQLENDL	Pen a 1 89-101	NRRIQLLEEDLER
Pen a 1 61-75	QESLLKANIQLVEKD	Pen a 1 45-56	NLQKRMQQLEND	Pen a 1 89-102	NRRIQLLEEDLERS
Pen a 1 67-81	ANIQLVEKDKALSNA	Pen a 1 46-57	LQKRMQQLENDL	Pen a 1 89-103	NRRIQLLEEDLERSE
Pen a 1 73-87	EKDKALSNAEGEVAA			Pen a 1 90-104	RRIQLLEEDLERSEE
Pen a 1 85-99	VAALNRRIQLLEEDL			Pen a 1 90-103	RRIQLLEEDLERSE
Pen a 1 91-105	RIQLLEEDLERSEER			Pen a 1 90-102	RRIQLLEEDLERS
Pen a 1 97-111	EDLERSEERLNTATT			Pen a 1 90-101	RRIQLLEEDLER
Pen a 1 103-117	EERLNTATTKLAEAS			Pen a 1 91-101	RIQLLEEDLER
Pen a 1 109-123	ATTKLAEASQAADES			Pen a 1 91-105	RIQLLEEDLERSEER
Pen a 1 115-129	EASQAADESERMRKV			Pen a 1 91-102	RIQLLEEDLERS
Pen a 1 121-135	DESERMRKVLENRSL			Pen a 1 92-101	IQLLEEDLER
Pen a 1 127-141	RKVLENRSLSDEERM			Pen a 1 92-102	IQLLEEDLERS
Pen a 1 133-147	RSLSDEERMDALENQ			Pen a 1 98-107	DLERSEERLN
Pen a 1 139-153	ERMDALENQLKEARF			Pen a 1 99-107	LERSEERLN
Pen a 1 145-159	ENQLKEARFLAEEAD			Pen a 1 99-109	LERSEERLNTA
Pen a 1 163-177	DEVARKLAMVEADLE			Pen a 1 103-113	EERLNTATTKL
Pen a 1 169-183	LAMVEADLERAEERA			Pen a 1 104-113	ERLNTATTKL
Pen a 1 175-189	DLERAEERAETGESK			Pen a 1 105-113	RLNTATTKL
Pen a 1 187-201	ESKIVELEEELRVVG			Pen a 1 105-114	RLNTATTKLA
Pen a 1 193-207	LEEELRVVGNNLKSL				
Pen a 1 211-225	EEKANQREEAYKEQI				
Pen a 1 217-231	REEAYKEQIKTLTNK				
Pen a 1 223-237	EQIKTLTNKLKAAEA				
Pen a 1 247-261	QKLQKEVDRLEDELV				
Pen a 1 253-267	VDRLEDELVNEKEKY				
Pen a 1 259-273	ELVNEKEKYKSITDE				
Pen a 1 265-279	EKYKSITDELDQTFS				
Pen a 1 270-284	ITDELDQTFSELSGY				

Table 2 (continiued)

IgE-reactive, peptides identified by SPOTSIZE of Pen a 1 region 3		IgE-reactive, peptides identified by SPOTSIZE of Pen a 1 region 4		IgE-reactive, peptides identified by SPOTSIZE of Pen a 1 region 5	
Pen a 1 126-133	MRKVLENR	Pen a 1 187-198	ESKIVELEEELR	Pen a 1 187-198	ESKIVELEEELR
Pen a 1 127-133	RKVLENR	Pen a 1 187-199	ESKIVELEEELRV	Pen a 1 187-199	ESKIVELEEELRV
Pen a 1 127-135	RKVLENRSL	Pen a 1 187-200	ESKIVELEEELRVV	Pen a 1 187-200	ESKIVELEEELRVV
Pen a 1 132-139	NRSLSDEE	Pen a 1 187-201	ESKIVELEEELRVVG	Pen a 1 187-201	ESKIVELEEELRVVG
Pen a 1 133-139	RSLSDEE	Pen a 1 188-201	SKIVELEEELRVVG	Pen a 1 188-201	SKIVELEEELRVVG
Pen a 1 133-141	RSLSDEERM	Pen a 1 189-199	KIVELEEELRV	Pen a 1 189-199	KIVELEEELRV
Pen a 1 133-146	RSLSDEERMDALEN	Pen a 1 189-200	KIVELEEELRVV	Pen a 1 189-200	KIVELEEELRVV
Pen a 1 133-147	RSLSDEERMDALENQ	Pen a 1 189-201	KIVELEEELRVVG	Pen a 1 189-201	KIVELEEELRVVG
Pen a 1 134-139	SLSDEE	Pen a 1 190-201	IVELEEELRVVG	Pen a 1 190 201	IVELEEELRVVG
Pen a 1 134-144	SLSDEERMDAL	Pen a 1 191-200	VELEEELRVV	Pen a 1 191-200	VELEEELRVV
Pen a 1 134-146	SLSDEERMDALEN	Pen a 1 191-201	VELEEELRVVG	Pen a 1 191-201	VELEEELRVVG
Pen a 1 134-147	SLSDEERMDALENQ	Pen a 1 193-201	LEEELRVVG	Pen a 1 193-201	LEEELRVVG
Pen a 1 135-143	LSDEERMDA	Pen a 1 193-202	LEEELRVVGN	Pen a 1 193-202	LEEELRVVGN
Pen a 1 135-144	LSDEERMDAL	Pen a 1 195-202	EELRVVGN	Pen a 1 195-202	EELRVVGN
Pen a 1 135-145	LSDEERMDALE	Pen a 1 195-203	EELRVVGNN	Pen a 1 195-203	EELRVVGNN
Pen a 1 135-146	LSDEERMDALEN	Pen a 1 195-206	EELRVVGNNLKS	Pen a 1 195-206	EELRVVGNNLKS
Pen a 1 135-147	LSDEERMDALENQ	Pen a 1 196-207	ELRVVGNNLKSL	Pen a 1 196-207	ELRVVGNNLKSL
Pen a 1 136-143	SDEERMDA	Pen a 1 197-207	LRVVGNNLKSL	Pen a 1 197-207	LRVVGNNLKSL
Pen a 1 139-153	ERMDALENQLKEARF	Pen a 1 199-205	VVGNNLK	Pen a 1 199-205	VVGNNLK
Pen a 1 140-153	RMDALENQLKEARF				
Pen a 1 141-149	MDALENQLK				
Pen a 1 141-153	MDALENQLKEARF				
Pen a 1 144-149	LENQLK				
Pen a 1 144-151	LENQLKEA				

Table 3.1 Combinatorial Substitutions Region 1				
	Spot	peptide	#12	#4
Hom a TMs*	1	1	[illegible]	[illegible]
	2	2	I...........	I...........
	3	3	.R..........	.R..........
	4	4	[illegible]	..I.........
	5	5	...T........	...T........
	6	6	....H.......	....H.......
	7	7	......K.....	......K.....
	8	8	..........V..	..........V..
	9	9	IR..........	IR..........
	10	10	[illegible]	I.I.........
	11	11	I..T........	I..T........
	12	12	I...H.......	I...H.......
	13	13	I.....K.....	I.....K.....
	14	14	I.........V..	I.........V..
	15	15	.RI.........	.RI.........
	16	16	.R.T........	.R.T........
	17	17	.R..H.......	.R..H.......
	18	18	.R....K.....	.R....K.....
	19	19	.R........V..	.R........V..
	20	20	..IT........	..IT........
	21	21	..I.H.......	..I.H.......
	22	22	..I...K.....	..I...K.....
	23	23	..I.......V..	..I.......V..
	24	24	...TH.......	...TH.......
	25	25	...T..K.....	...T..K.....
	26	26	...T......V..	...T......V..
	27	27	....H.K.....	....H.K.....
	28	28	....H.....V..	....H.....V..
	29	29	......K...V..	......K...V..
	30	30	IRITH.K...V..	IRITH.K...V..
Per a 7*	37	31	VHELQKRMQQLEN	VHELQKRMQQLEN
	38	32	A...........	A...........
	39	33	.R..........	.R..........
	40	34	[illegible]	..S.........
	41	35	......K.....	......K.....
	42	36	[illegible]	[illegible]
	43	37	..........I..	..........I..
	44	38	AR..........	AR..........
	45	39	A.S.........	A.S.........
	46	40	A.....K.....	A.....K.....
	47	41	A......I.....	[illegible]
	48	42	A.........I..	A.........I..
	49	43	.RS.........	.RS.........
	50	44	.R....K.....	.R....K.....
	51	45	.R.....I.....	.R.....I.....
	52	46	.R........I..	.R........I..
	53	47	..S...K.....	..S...K.....
	54	48	[illegible]	[illegible]
	55	49	..S.......I..	[illegible]
	56	50	......KI.....	[illegible]
	57	51	......K...I..	......K...I..
	58	52	.......I..I..	[illegible]
	59	53	ARS...KI..I..	ARS...KI..I..

Table 3.1
Combinatorial Substitutions
Region 1

	Spot	peptide	#12	#4
Hom a TMs*	1	1	VHNLQKRMQQLEN	VHNLQKRMQQLEN
	2	2	I............	I............
	3	3	.R...........	.R...........
	4	4	[illegible]	..I..........
	5	5	...T.........	...T.........
	6	6	....H........	....H........
	7	7	......K......	......K......
	8	8	..........V..	..........V..
	9	9	IR...........	IR...........
	10	10	I.I..........	I.I..........
	11	11	I..T.........	I..T.........
	12	12	I...H........	I...H........
	13	13	I.....K......	I.....K......
	14	14	I.........V..	I.........V..
	15	15	.RI..........	.RI..........
	16	16	.R.T.........	.R.T.........
	17	17	.R..H........	.R..H........
	18	18	.R....K......	.R....K......
	19	19	.R........V..	.R........V..
	20	20	..IT.........	..IT.........
	21	21	..I.H........	..I.H........
	22	22	..I...K......	..I...K......
	23	23	..I.......V..	..I.......V..
	24	24	...TH........	...TH........
	25	25	...T..K......	...T..K......
	26	26	...T......V..	...T......V..
	27	27	....H.K......	....H.K......
	28	28	....H.....V..	....H.....V..
	29	29	......K...V..	......K...V..
	30	30	IRITH.K...V..	IRITH.K...V..
Per a 7*	37	31	VHNLQKRMQQLEN	VHNLQKRMQQLEN
	38	32	A............	A............
	39	33	.R...........	.R...........
	40	34	..S..........	..S..........
	41	35	......K......	......K......
	42	36	.......I.....	.......I.....
	43	37	..........I..	..........I..
	44	38	AR...........	AR...........
	45	39	A.S..........	A.S..........
	46	40	A.....K......	A.....K......
	47	41	A......I.....	A......I.....
	48	42	A.........I..	A.........I..
	49	43	.RS..........	.RS..........
	50	44	.R....K......	.R....K......
	51	45	.R.....I.....	.R.....I.....
	52	46	.R........I..	.R........I..
	53	47	..S...K......	..S...K......
	54	48	..S....I.....	..S....I.....
	55	49	..S.......I..	..S.......I..
	56	50	......KI.....	......KI.....
	57	51	......K...I..	......K...I..
	58	52	.......I..I..	.......I..I..
	59	53	ARS...KI..I..	ARS...KI..I..

Table 3.1
Combinatorial Substitutions
Region 1 (continued)

	Spot	peptide	#12	#4
Der p 10*	61	54	VHNLQKRMQQLEN	VHNLQKRMQQLEN
	62	55	.R..........	.R...........
	63	56	[illegible]	[illegible]
	64	57	......K......	......K......
	65	58	[illegible]	[illegible]
	66	59	..........I..	..........I..
	67	60	.RA..........	.RA..........
	68	61	.R...K......	.R...K......
	69	62	.R.....I.....	.R.....I.....
	70	63	.R........I..	.R........I..
	71	64	..A...K......	..A...K......
	72	65	[illegible]	[illegible]
	73	66	..A.......I..	[illegible]
	74	67	......KI.....	[illegible]
	75	68	......K...I..	......K...I..
	76	69	.......I..I..	[illegible]
	77	70	.RA...KI..I..	.RA...KI..I..

Table 3.1
Combinatorial Substitutions
Region 1 (continued)

	Spot	peptide	#12	#4
	97	71	LVHNLQKRMQQLEN	VHNLQKRMQQLEN
	98	72	L............	L............
	99	73	.V...........	.V...........
	100	74	[illegible]	[illegible]
	101	75	......K......	......K......
	102	76	[illegible]	[illegible]
	103	77	........K....	........K....
	104	78	.........G...	.........G....
	105	79	..........T..	..........T..
	106	80	............D	............D
	107	81	LV...........	LV...........
	108	82	L.A..........	L.A..........
	109	83	L....K......	L....K......
	110	84	L......L.....	L......L.....
	111	85	L.......K....	L.......K....
	112	86	L........G...	L........G...
	113	87	L.........T..	L.........T..
	114	88	L...........D	L..........D
	115	89	.VA..........	.VA..........
	116	90	.V....K......	.V....K......
	117	91	.V.....L.....	.V.....L.....
	118	92	.V......K....	.V......K....
	119	93	.V.......G...	.V.......G...
chicken α-TM*	120	94	.V........T..	.V........T..
	121	95	.V..........D	.V.........D
	122	96	..A...K......	..A...K......
	123	97	..A....L.....	..A....L.....
	124	98	..A.....K....	..A.....K....
	125	99	..A......G...	..A......G...
	126	100	..A.......T..	..A.......T..
	127	101	..A.........D	..A.........D
	128	102	......KL.....	......KL.....
	129	103	......K.K....	......K.K....
	130	104	......K..G...	......K..G...
	131	105	......K...T..	......K...T..
	132	106	......K.....D	......K.....D
	133	107	.......LK....	.......LK....
	134	108	.......L.G...	.......L.G...
	135	109	.......L..T..	.......L..T..
	136	110	.......L....D	.......L....D
	137	111	........KG...	........KG...
	138	112	........K.T..	........K.T..
	139	113	........K...D	........K...D
	140	114	.........GT..	.........GT..
	141	115	.........G..D	.........G..D
	142	116	..........T.D	..........T.D
	143	117	LVA...KLKGT.D	LVA...KLKGT.D
rabbit α-TM*	144	118	..S......G...	..S......G...
	145	119	LVS...KLKGT.D	LVS...KLKGT.D

Table 3.1 Combinatorial Substitutions Region 1 (continued)				
	Spot	peptide	#12	#4
chicken β-TM*	146	120	Q...........	Q...........
	157	121	.Q..........	.Q..........
	158	122	..G.........	..G.........
	159	123	QQ..........	QQ..........
	160	124	Q.G.........	Q.G.........
	161	125	Q....K......	Q....K......
	162	126	Q.....L.....	Q.....L.....
	163	127	Q......K....	Q......K....
	164	128	Q.......G...	Q.......G...
	165	129	Q........T..	Q........T..
	166	130	Q..........D	Q..........D
	167	131	.QG.........	.QG.........
	168	132	.Q...K......	.Q...K......
	169	133	.Q....L.....	.Q....L.....
	170	134	.Q.....K....	.Q.....K....
	171	135	.Q......G...	.Q......G...
	172	136	.Q.......T..	.Q.......T..
	173	137	.Q.........D	.Q.........D
	174	138	..G..K......	..G..K......
	175	139	..G...L.....	..G...L.....
	176	140	..G....K....	..G....K....
	177	141	..G.....G...	..G.....G...
	178	142	..G......T..	..G......T..
	179	143	..G........D	..G........D
	180	144	QQG...KLKGT.D	QQG...KLKGT.D
swineTM*	181	145	..S.........	..S.........
	193	146	.........A...	.........A...
	194	147	L.S.........	L.S.........
	195	148	L........A...	L........A...
	196	149	.VS.........	.VS.........
	197	150	.V.......A...	.V.......A...
	198	151	..S..K......	..S..K......
	199	152	..S...L.....	..S...D.....
	200	153	..S....K....	..S....K....
	201	154	..S.....A...	..S.....A...
	202	155	..S......T..	..S......T..
	203	156	..S........D	..S........D
	204	157	.....K..A...	.....K..A...
	205	158	......L.A...	......L.A...
	206	159	........KA...	........KA...
	207	160	.........AT..	.........AT..
	208	161	.........A..D	.........A..D
	209	162	LVS...KLKAT.D	LVS...KLKAT.D
rabbit β-TM*	210	163	Q.A.........	Q.A.........
	217	164	.QA.........	.QA.........
	218	165	QQA...KLKGT.D	QQA...KLKGT.D

Table 3.1 Combinatorial Substitutions Region 1 (continued)				
	Spot	peptide	#12	#4
salmonTM1*	219	166	.I..........	.I..........
	229	167	..Q.........	..Q.........
	230	168	...M........	...M........
	231	169	LI..........	LI..........
	232	170	L.Q.........	L.Q.........
	233	171	L..M........	L..M........
	234	172	.IQ.........	.IQ.........
	235	173	.I.M........	.I.M........
	236	174	.I....K......	.I....K......
	237	175	.I.....L.....	.I.....L.....
	238	176	.I......K....	.I......K....
	239	177	.I.......G...	.I.......G...
	240	178	.I........T..	.I........T..
	241	179	.I..........D	.I..........D
	242	180	..QM.........	..QM.........
	243	181	..Q...K......	..Q...K......
	244	182	..Q...L.....	..Q...L.....
	245	183	..Q.....K....	..Q.....K....
	246	184	..Q......G...	..Q......G...
	247	185	..Q.......T..	..Q.......T..
	248	186	..Q.........D	..Q.........D
	249	187	...M..K......	...M..K......
	250	188	...M...L.....	...M...L.....
	251	189	...M....K....	...M....K....
	252	190	...M.....G...	...M.....G...
	253	191	...M......T..	...M......T..
	254	192	...M........D	...M........D
	255	193	LIQM..KLKGT.D	LIQM..KLKGT.D
salmonTM2*	256	194	.L...........	.L...........
	265	195	......N......	......N......
	266	196	LL...........	LL...........
	267	197	L.....N......	L.....N......
	268	198	.LS..........	.LS..........
	269	199	.L....N......	.L....N......
	270	200	.L.....L.....	.L.....L.....
	271	201	.L......K....	.L......K....
	272	202	.L.......G...	.L.......G...
	273	203	.L........T..	.L........T..
	274	204	.L..........D	.L..........D
	275	205	..S...N......	..S...N......
	276	206	......NL.....	......NL.....
	277	207	......N.K....	......N.K....
	278	208	......N..G...	......N..G...
	279	209	......N...T..	......N...T..
	280	210	......N.....D	......N.....D
	281	211	LLS...NLKGT.D	LLS...NLKGT.D

*: mutated peptides resulting from sequence differences of Pen a 1 with this tropomosin.

Table 3.2
Combinatorial substitutions
region 2

	Spot	peptide	#6	#10	#18 (not done)	Spot	peptide	#12
Der p 10	1	1	ALNRRIQLLEEDLER	ALNRRIQLLEEDLER	ALNRRIQLLEEDLER	1	1	RIQLLEEDLER
	2	2	........I......	........I......	........I......	2	2	....I......
GalgoTM*	13	3	ALNRRIQLLEEDLER	ALNRRIQLLEEDLER	ALNRRIQLLEEDLER	3	13	[illegible]
	14	4	S..............	S..............	S..............			
	15	5	........V......	........V......	........V......	4	14	....V......
	16	6	...........E...	...........E...	...........E...	5	15	.......E...
	17	7	.............D.	.............D.	.............D.	6	16	[illegible]D.
	18	8	S.......V......	S.......V......	S.......V......			
	19	9	S..........E...	S..........E...	S..........E...			
	20	10	S............D.	S............D.	S............D.			
	21	11	........V..E...	........V..E...	........V..E...	7	17	....V..E...
	22	12	........V....D.	........V....D.	........V....D.	8	18	....V....D.
	23	13	...........E.D.	...........E.D.	...........E.D.	9	19	.......E.D.
	24	14	S.......V..E...	S.......V..E...	S.......V..E...			
	25	15	S.......V....D.	S.......V....D.	S.......V....D.			
	26	16	S..........E.D.	S..........E.D.	S..........E.D.			
	27	17	........V..E.D.	........V..E.D.	........V..E.D.	10	20	....V..E.D.
	28	18	S.......V..E.D.	S.......V..E.D.	S.......V..E.D.			
SussxTM*	29	19	........F......	........F......	........F......	11	21	....F......
	30	20	S.......F......	S.......F......	S.......F......			
	31	21	........F..E...	........F..E...	........F..E...	12	22	....F..E...
	32	22	........F....D.	........F....D.	........F....D.	13	23	....F....D.
	33	23	S.......F..E...	S.......F..E...	S.......F..E...			
	34	24	S.......F....D.	S.......F....D.	S.......F....D.			
	35	25	........F..E.D.	........F..E.D.	........F..E.D.	14	24	....F..E.D.
	36	26	S.......F..E.D.	S.......F..E.D.	S.......F..E.D.			
SalrTM2*	37	27	G..............	G..............	G..............			
	38	28	G.......V......	G.......V......	G.......V......			
	39	29	G..........E...	G..........E...	G..........E...			
	40	30	G............D.	G............D.	G............D.			
	41	31	G.......V..E...	G.......V..E...	G.......V..E...			
	42	32	G.......V....D.	G.......V....D.	G.......V....D.			
	43	33	G..........E.D.	G..........E.D.	G..........E.D.			
	44	34	G.......V..E.D.	G.......V..E.D.	G.......V..E.D.			

*: mutated peptides resulting from sequence differences of Pen a 1 with this tropomyosin.

Table 3.3
Combinatorial Substitutions
region 3 (epitope 3a)

	#12			#1			#5			#18			#10
	spot	peptide		spot	peptide		spot	peptide		spot	peptide		
Per a 7	1	1	RSLSDEERM	1	1	LSDEERMDALEN	1	1	SDEERMDA				
	2	2	K[illegible]										
	3	3	.G.......										
	4	4	[illegible]A[illegible]	2	2	.A..........	2	2	A.......				
	5	5	KG.......										
	6	6	[illegible]K.A[illegible]										
	7	7	.G.A.....										
	8	8	KG.A.....										
Der p10	13	9	RSLSDEERM	13	3	LSDEERMDALEN	13	3	SDEERMDA	1	1	[illegible]DEERMDALE[illegible]	[illegible]DEERMDALE[illegible]
	14	10	..I......	14	4	I...........							
	15	11	...T.....	15	5	.T[illegible]	14	4	T.......				
				16	6	.......E....	15	5	......E.	2	2	.....E...	.....E...
				17	7	........G...	16	6	.......G	3	3	......G..	......G..
	16	12	..IT.....	18	8	IT..........							
				19	9	I......E....							
				20	10	I.......G...							
				21	11	.T.....E....	17	7	T.....E.				
				22	12	.T......G...	18	8	T......G				
				23	13	.......EG...	19	9	......EG	4	4	.....EG..	.....EG..
				24	14	IT.....E....							
				25	15	IT......G...							
				26	16	I......EG...							
				27	17	.T.....EG...	20	10	T.....EG				
				28	18	IT.....EG...							

Table 3.3
Combinatorial Substitutions
region 3 (epitope 3a (continued)

	#12			#1			#5			#18			#10
	spot	peptide		spot	peptide		spot	peptide		spot	peptide		
	25	13	RSLSDEERM	37	19	RLSDEERMDALEN(G	25	11	SDEERMDA	13	5	DEERMDALE	DEERMDALE
		14	[illegible]										
	26	15	..Q......	38	20	Q...........							
	27	16	...K.....	39	21	.K..........	26	12	K.......				
	28	17	[illegible]K[illegible]	40	22	.....K......	27	13	....K...	14	6	...K.....	...K.....
				41	23	.......E....	28	14	......E.	15	7	.....E...	.....E...
				42	24	[illegible]	29	15	I	16	8	......I..	[illegible]
				43	25	.........Q..				17	9	[illegible]	[illegible]
				44	26	I							
	29	18	.AQ......										
	30	19	.A.K.....										
	31	20	.A.....K.										
	32	21	..QK.....	45	27	QK.........							
	33	22	..Q....K.	46	28	Q....K......							
				47	29	Q......E....							
				48	30	Q.......I...							
				49	31	Q........Q..							
				50	32	Q..........I							
	34	23	...K...K.	51	33	.K...K......	30	16	K...K...				
				52	34	.K.....E....	31	17	K.....E.				
				53	35	.K.......I...	32	18	K......I				
				54	36	.K........Q..							
				55	37	.K..........I							
GalgαTM*				56	38	.....K.E....	33	19	....K.E.	18	10	...K.E...	...K.E...
				57	39	[illegible]K.I[illegible]	34	20	....K..I	19	11	...K..I..	...K..I..
				58	40	.....K...Q..				20	12	...K...Q.	...K...Q.
				59	41	.....K.....I							
				60	42	.......EI...	35	21	......EI	21	13	.....EI..	.....EI..
				61	43	.......E.Q..				22	14	.....E.Q.	.....E.Q.
				62	44	.......E...I							
				63	45	........IQ..							
				64	46	[illegible]I.E[illegible]				23	15	......IQ.	[illegible]IQ[illegible]
				65	47	.........Q.I							
	35	24	.AQK.....										
	36	25	.AQ....K.										
	37	26	.A.K...K.										
	38	27	..QK...K.										
	39	28	.AQK...K.										
				66	48	QK...K.EIQ.I							
							36	22	K...K.E.				
							37	23	K...K..I				
							38	24	K.....EI	24	16	...K.EI..	...K.EI..
							39	25	....K.EI				
							40	26	K...K.EI				
										25	17	...K.E.Q.	...K.E.Q.
										26	18	...K..IQ.	...K..IQ.
										27	19	.....EIQ.	.....EIQ.
										28	20	...K.EIQ.	...K.EIQ.

Table 3.3
Combinatorial Substitutions
region 3 (epitope 3a) (continued)

	#12			#1			#5			#18			#10
	spot	peptide		spot	peptide		spot	peptide		spot	peptide		
Galg βTM*	49	29	..M......	73	49	[illegible]	49	27	L	37	21	......L..	......L..
				74	50	[illegible]							
				75	51	[illegible]							
	50	30	.AM......										
	51	31	..MK.....	76	52	MK..........							
	52	32	..M....K.	77	53	M....K......							
				78	54	M......B....							
				79	55	[illegible]							
				80	56	M........Q..							
				81	57	[illegible]							
				82	58	.K......L...	50	28	K......L				
				83	59	.K.........M							
				84	60	[illegible]	51	29	....K..L	38	22	...K..L..	...K..L..
				85	61	.....K.....M							
				86	62	.......EL...	52	30	......EL	39	23	.....EL..	.....EL..
				87	63	.......E...M							
				88	64	........LQ..				40	24	......LQ.	......LQ.
				89	65	[illegible]							
				90	66	.........Q.M							
	53	33	.AMK.....										
	54	34	.AM....K.										
	55	35	..MK...K.										
							53	31	K...K..L				
							54	32	K.....EL				
							55	33	....K.EL	41	25	...K.EL..	...K.EL..
										42	26	...K..LQ.	...K..LQ.
										43	27	.....ELQ.	.....ELQ.
	56	36	.AMK...K.										
							56	34	K...K.EL				
										44	28	...K.ELQ.	...K.ELQ.
				91	67	MK...K.ELQ.M							

Table 3.3
Combinatorial Substitutions
region 3 (epitope 3a) (continued)

	#12			#1			#5			#18			#10
	spot	peptide		spot	peptide		spot	peptide		spot	peptide		
	61	37	..S......	97	68	S...........							
				98	69	..........D.				49	29	[illegible]	[illegible]
	62	38	.AS......										
	63	39	..SK.....	99	70	SK..........							
	64	40	..S....K.	100	71	S....K......							
				101	72	S......E....							
				102	73	S.......L...							
				103	74	S........Q..							
				103	75	S.........D.							
				105	76	S..........I							
				106	77	.K........D.							
				107	78	.....K....D.				50	30	...K....D	...K....D
				108	79	........E..D.				51	31	......E..D	......E..D
				109	80	[illegible]				52	32	.......L.D	.......L.D
				110	81	[illegible]							
				111	82	.........QD.				53	34	[illegible]	[illegible]
SaltTM1*				112	83	..........DI							
	65	41	.ASK.....										
	66	42	.AS....K.										
	67	43	..SK...K.										
	68	44	.ASK...K.										
										54	35	...K.E..D	...K.E..D
										55	36	...K..L.D	...K..L.D
										56	37	...K...QD	...K...QD
										57	38	.....EL.D	.....EL.D
										58	39	......E.QD	.....E.QD
										59	40	......LQD	......LQD
										60	41	...K.EL.D	...K.EL.D
										61	42	...K.E.QD	...K.E.QD
										62	43	...K..LQD	...K..LQD
										63	44	.....ELQD	.....ELQD
										64	45	...K.ELQD	...K.ELQD
				113	84	SK...K.ELQDI							

*: mutated peptides resulting from sequence differences of Pen a 1 with this tropomosin.

Table 3.4
Combinatorial Substitutions
region 3 (epitope 3b)

	#4			#6		
	spots	peptide		spots	peptide	
Der p 10*	1	1				
	2	2				
	3	3	....G.........			
	4	4	..............M			
	5	5	...EG.........			
	6	6	...E.........M			
	7	7	....G........M			
	8	8	...EG........M			
GalgαTM*	13	9		1	1	LENQLKEA
	14	10				
	15	11				
	16	12				
	17	13	.....Q........	2	2	Q.......
	18	14	.......I.......	3	3	..I.....
	19	15	.............K.			
	20	16	..............H			
	21	17				
	22	18				
	23	19	.K...Q........			
	24	20				
	25	21				
	26	22	.K............H			
	27	23				
	28	24	...E.Q........			
	29	25	...E...I.......			
	30	26				
	31	27	...E..........H			
	32	28	....IQ.........			
	33	29	....I..I.......			
	34	30				
	35	31	....I.........H			
	36	32	.....Q.I.......	4	4	Q.I.....
	37	33	.....Q.......K.			
	38	34	.....Q........H			
	39	35	.......I.....K.			
	40	36	.......I......H			
	41	37	.............KH			
	42	38	.K.EIQ.I.....KH			
GalgβTM*	49	39				
	50	40	.......M.......	13	5	..M.....
	51	41				
	52	42				
	53	43				
	54	44	...S...M.......			
	55	45				
	56	46	....L..M.......			
	57	47				
	58	48	....L.........H			
	59	49	.....Q.M.......	14	6	Q.M.....
	60	50	.......M.....K.			
	61	51	.......M......H			
	62	52	.K.ELQ.M.....KH			
SaltTM1*	73	53	......D........	25	7	.D......
	74	54	.K....D........	26	8	QD......
	75	55	...E..D........	27	9	.DI.....
	76	56	....L.D........	28	10	QDI.....
	77	57	....L..I.......			
	78	58	.....QD........			
	79	59	......DI.......			
	80	60	......D......K.			
	81	61	......D.......H			
	82	62	.K.ELQDI.....KH			

*: mutated peptides resulting from sequence differences of Pen a 1 with this tropomyosin

Table 3.5
Combinatorial Substitution Analysis
Epitope 5a

	spot	peptide	#2	spot	peptide	#4	spot	peptide	#10	spot	peptide	#18
	13	1	LQKEVDRLEDEL	25	1	[illegible]KEVDRLEDE[illegible]	13	1	LQKEVDRLEDELV			
	14	2	.E..........				14	2	.E...........			
	15	3	...S........	26	2	[illegible]	15	3	...S.........			
	15	4	....I.......	27	3	[illegible]	16	4	[illegible]			
	17	5	......D.....	28	4	....D....	17	5	......D......			
							18	6	............Y			
	18	6	.E.S........				19	7	.E.S.........			
	19	7	.E..I.......				20	8	.E..I........			
	20	8	.E....D.....				21	9	.E....D......			
							22	10	.E..........Y			
	21	9	...SI.......	29	5	[illegible]	23	11	...SI........			
	22	10	...S..D.....	30	6	.S..D....	24	12	...S..D......			
							25	13	...S........Y			
	23	11	....I.D.....	31	7	..I.D....	26	14	....I.D......			
							27	15	[illegible]			
							28	16	......D.....Y			
Galg αTM*	24	12	.E.SI.......				29	17	.E.SI........			
	25	13	.E.S..D.....				30	18	.E.S..D......			
							31	19	.E.S........Y			
							32	20	.E..I.D......			
							33	21	.E..I.......Y			
							34	22	.E....D.....Y			
							35	23	...SI.D......			
							36	24	...SI.......Y			
							37	25	...S..D.....Y			
							38	26	....I.D.....Y			
							39	27	.E.SI.D......			
							40	28	.E.SI.......Y			
							41	29	.E.S..D.....Y			
	26	14	.E..I.D.....				42	30	.E..I.D.....Y			
	27	15	...SI.D.....	32	8	.SI.D....	43	31	...SI.D.....Y			
	28	16	.E.SI.D.....				44	32	.E.SI.D.....Y			

Table 3.5 (continued)
Combinatorial Substitution Analysis
Epitope 5a

	spot	peptide	#2	spot	peptide	#4	spot	peptide	#10	spot	peptide	#18
	37	17	...T........	37	9	[illegible]	49	33	...T.........			
	38	18	V				50	34	...........V.			
	39	19	.E.T........				51	35	.E.T.........			
	40	20	.E.........V				52	36	.E...... ..V.			
	41	21	...TI.......	38	10	...TI......	53	37	...TI........			
	42	22	...T..D.....	39	11	.T..D....	54	38	...T..D......			
	43	23	...T.......V				55	39	...T.......V.			
	44	24	....I......V				56	40	...T........Y			
	45	25	......D....V				57	41	....I......V.			
							58	42	......D....V.			
							59	43	...........VY			
	46	26	.E.TI.......				60	44	.E.TI........			
	47	27	.E.T..D.....				61	45	.E.T..D......			
	48	28	.E.T.......V				62	46	.E.T.......V.			
	49	29	.E..I......V				63	47	.E.T........Y			
							64	48	.E..I......V.			
	50	30	.E....D....V				65	49	.E....D....V.			
							66	50	.E.........VY			
	51	31	...TI.D.....	40	12	.TI.D....	67	51	...TI.D......			
	52	32	...TI......V				68	52	...TI......V.			
							69	53	...TI.......Y			
	53	33	...T..D....V				70	54	...T..D....V.			
Galg βTM*							71	55	...T..D.....Y			
							72	56	...T.......VY			
	54	34	....I.D....V				73	57	....I.D....V.			
							74	58	....I......VY			
							75	59	......D....VY			
	55	35	.E.TI.D.....				76	60	.E.TI.D......			
	56	36	.E.TI......V				77	61	.E.TI......V.			
							78	62	.E.TI.......Y			
	57	37	.E.T..D....V				79	63	.E.T..D....V.			
							80	64	.E.T..D.....Y			
							81	65	.E.T.......VY			
	58	38	.E..I.D....V				82	66	.E..I.D....V.			
							83	67	.E..I......VY			
							84	68	.E....D....VY			
	59	39	...TI.D....V				85	69	...TI.D....V.			
							86	70	...TI.D.....Y			
							87	71	...TI......VY			
							88	72	...T..D....VY			
							89	73	....I.D....VY			
							90	74	.E.TI.D....V.			
	60	40	.E.TI.D....V				91	75	.E.TI.D.....Y			
							92	76	.E.TI......VY			
							93	77	.E.T..D....VY			
							94	78	.E..I.D....VY			
							95	79	...TI.D....VY			
							96	80	.E.TI.D....VY			

Table 3.5 (continued)
Combinatorial Substitution Analysis
Epitope 5b

	spot	peptide	#2	spot	peptide	#4	spot	petide	#10	spot	peptide	#18
Per a 7*	1	41	[illegible]	1	13	[illegible]	1	81	[illegible]	1	1	[illegible]
	2	42	...F....	2	14	...F....	2	82	...F....	2	2	...F....
	3	43	[illegible]	3	15	[illegible]	3	83	[illegible]	3	3	[illegible]
	4	44	[illegible]	4	16	[illegible]	4	84	[illegible]	4	4	[illegible]
	5	45	...F.C..	5	17	...F.C..	5	85	...F.C..	5	5	...F.C..
	6	46	...F...D	6	18	...F...D	6	86	...F...D	6	6	...F...D
	7	47	[illegible]	7	19	[illegible]	7	87	[illegible]	7	7	[illegible]
	8	48	...F.C.D	8	20	...F.C.D	8	88	...F.C.D	8	8	...F.C.D
Der p 10*	11	49	[illegible]	11	21	[illegible]	11	89	[illegible]	11	9	[illegible]
	12	50	[illegible]	12	22	[illegible]	12	90	[illegible]	12	10	[illegible]
Galg αTM*	61	51	[illegible]	49	23	[illegible]	97	91	[illegible]	13	11	[illegible]
	62	52	...A....	50	24	...A....	98	92	[illegible]	14	12	...A....
	63	53	[illegible]	51	25	[illegible]	99	93	[illegible]	15	13	[illegible]
	64	54	[illegible]	52	26	[illegible]	100	94	[illegible]	16	14	[illegible]
	65	55	...A.S..	53	27	[illegible]	101	95	[illegible]	17	15	...A.S..
	66	56	...A..E.	54	28	[illegible]	102	96	...A..E.	18	16	...A..E.
	67	57	[illegible]	55	29	.....SE.	103	97	[illegible]	19	17	[illegible]
	68	58	...A.SE.	56	30	...A.SE.	104	98	...A.SE.	20	18	...A.SE.

Table 3.5 (continued)
Combinatorial Substitution Analysis
Epitope 5c

	spot	peptide	#2	spot	peptide	#4	spot	petide	#10	spot	peptide	#18
				13	31	[illegible] ELDQTFSEL [illegible]						
				14	32	[illegible] D [illegible]						
				15	33	[illegible]						
Per a 7*				16	34	[illegible]						
				17	35	[illegible] D [illegible] M [illegible]						
				18	36	[illegible]						
				19	37	...M..T..						
				20	38	D..M..T..						
Der p 10*				23	39	[illegible] ELDQTFAEL [illegible]						
				24	40	[illegible]						
				61	41	[illegible] ELDQTFSEL [illegible]						
				62	42	[illegible]						
				63	43	....A....						
				64	44	.....L...						
				65	45	[illegible] N [illegible]						
				66	46	.......D.						
				67	47	........M						
				68	48	...HA....						
				69	49	...H.L...						
				70	50	...H.N...						
				71	51	...H...D.						
Galg αTM*				72	52	...H....M						
				73	53	....AL...						
				74	54	....A.N..						
				75	55	....A..D.						
				76	56	....A...M						
				77	57	.....LN..						
				78	58	.....L.D.						
				79	59	.....L..M						
				80	60	......ND.						
				81	61	......N.M						
				82	62	.......DM						
				83	63	...HALNDM						
				85	64	[illegible] N [illegible]						
				86	65	I						
				87	66	...NA....						
				88	67	...N.L...						
				89	68	...N.N...						
				90	69	...N...D.						
SalfTM1*				91	70	...N....I						
				92	71	....A...I						
				93	72	.....L..I						
				94	73	......N.I						
				95	74	.......DI						
				96	75	...NALNDI						
				97	76	...N....M						
				98	77	...HALNDM						

*: mutated peptides resulting from sequence differences of Pen a 1 with this tropomyosin.

	Pen a 1		Region 1		Region 2		Region 3		Region 4		Region 5	
tropomyosin	Ide	Sim	Ide	Sim	Ide	Sim	Ide	Sim	Ide	Sim	Ide	Sim
Hom a TMf	98	98	100	100	100	100	100	100	100	100	100	100
Hom a Tms	93	96	46	80	100	100	100	100	100	100	100	100
Pan s 1	98	98	100	100	100	100	100	100	95	95	100	100
Per a 7	82	90	60	93	100	100	80	87	100	100	81	92
Der f 10	81	89	60	86	95	100	80	100	100	100	89	94
Der p 10	81	89	60	86	95	100	73	100	100	100	89	94
Dro m TM	70	87	46	93	100	100	87	87	95	100	68	81
Myt e TM	57	75	53	73	52	80	40	53	57	80	76	86
Onc v TM	70	83	53	80	80	90	53	73	90	95	81	94
Sch m TM	60	74	26	601	71	76	53	67	90	90	65	86
Gal g TM	58	72	33	53	71	85	47	67	76	85	44	63
Ory c TM	56	72	33	60	71	85	47	67	71	80	44	63

Table 4: Sequence identities (Ide) and similarities (Sim) among Pen a 1, Pen a 1 epitopes and and other allergenic and non-allergenic tropomyosins (TM)

Table 4

Table 5 (a)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 1			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 43-55	VHNLQKRMQQLEN	Pen a 1 43-55	VHNLQKRMQQLEN
mutated sequences containing 1 substitution	Pen a 1 44^{I}	.I...........	Pen a 1 43^{L}	L............
	Pen a 1 46^{N}	...N.........	Pen a 1 43^{I}	I............
	Pen a 1 46^{T}	...T.........	Pen a 1 44^{R}	.R...........
	Pen a 1 47^{H}	....H........	Pen a 1 44^{Q}	.Q...........
	Pen a 1 53^{V}	..........V..	Pen a 1 45^{S}	..S..........
	Pen a 1 43^{A}	A............	Pen a 1 45^{I}	..I..........
	Pen a 1 44^{V}	.V...........	Pen a 1 45^{G}	..G..........
	Pen a 1 51^{K}	........K....	Pen a 1 49^{K}	......K......
	Pen a 1 53^{T}	..........T..	Pen a 1 52^{G}	.........G...
	Pen a 1 44^{R}	.R...........	Pen a 1 52^{A}	.........A...
	Pen a 1 49^{K}	......K......	Pen a 1 53^{I}	..........I..
	Pen a 1 44^{L}	.L...........	Pen a 1 55^{D}	............D
	Pen a 1 49^{N}	......N......		
	Pen a 1 43^{Q}	Q............		
mutated sequences containing 2 substitutions	Pen a 1 43^{I} 44^{R}	IR...........	Pen a 1 43^{Q} 45^{A}	Q.A..........
	Pen a 1 43^{I} 46^{T}	I..T.........	Pen a 1 43^{Q} 50^{L}	Q......L.....
	Pen a 1 43^{I} 47^{H}	I...H........	Pen a 1 43^{L} 45^{S}	L.S..........
	Pen a 1 43^{I} 53^{V}	I.........V..	Pen a 1 43^{L} 45^{Q}	L.Q..........
	Pen a 1 44^{R} 45^{I}	.RI..........	Pen a 1 43^{L} 45^{A}	L.A..........
	Pen a 1 44^{R} 46^{T}	.R.T.........	Pen a 1 43^{L} 50^{L}	L......L.....
	Pen a 1 44^{R} 47^{H}	.R..H........	Pen a 1 43^{L} 52^{A}	L........A...
	Pen a 1 44^{R} 49^{K}	.R....K......	Pen a 1 43^{L} 55^{D}	L...........D
	Pen a 1 44^{R} 53^{V}	.R........V..	Pen a 1 43^{I} 45^{I}	I.I..........
	Pen a 1 45^{I} 46^{T}	..IT.........	Pen a 1 43^{I} 49^{K}	I.....K......
	Pen a 1 45^{I} 47^{H}	..I.H........	Pen a 1 43^{A} 50^{I}	A......I.....
	Pen a 1 45^{I} 53^{V}	..I.......V..	Pen a 1 44^{V} 50^{L}	.V.....L.....
	Pen a 1 46^{T} 47^{H}	...TH........	Pen a 1 44^{Q} 50^{L}	.Q.....L.....
	Pen a 1 46^{T} 49^{K}	...T..K......	Pen a 1 44^{L} 49^{N}	.L....N......

Table 5 (a)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 1 (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolishedIgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a I sequence	Pen a 1 43-55	VHNLQKRMQQLEN	Pen a 1 43-55	VHNLQKRMQQLEN
mutated sequences containing 2 substitutions	Pen a 1 46^{T} 53^{V}	...T......V..	Pen a 1 44^{L} 50^{L}	.L.....L.....
	Pen a 1 47^{H} 49^{K}	....H.K......	Pen a 1 45^{S} 49^{K}	..S...K......
	Pen a 1 47^{H} 53^{V}	....H.....V..	Pen a 1 45^{S} 52^{G}	..S......G...
	Pen a 1 49^{K} 53^{V}	......K...V..	Pen a 1 45^{S} 52^{A}	..S......A...
	Pen a 1 43^{A} 44^{R}	AR...........	Pen a 1 45^{S} 53^{I}	..S.......I..
	Pen a 1 43^{A} 45^{S}	A.S..........	Pen a 1 45^{Q} 49^{K}	..Q...K......
	Pen a 1 43^{A} 49^{K}	A.....K......	Pen a 1 45^{Q} 51^{K}	..Q.....K....
	Pen a 1 43^{A} 53^{I}	A.........I..	Pen a 1 45^{Q} 52^{G}	..Q......G...
	Pen a 1 44^{R} 45^{S}	.RS..........	Pen a 1 45^{I} 49^{K}	..I...K......
	Pen a 1 44^{R} 49^{K}	.R....K......	Pen a 1 45^{G} 49^{K}	..G...K......
	Pen a 1 44^{R} 50^{I}	.R.....I.....	Pen a 1 45^{A} 49^{K}	..A...K......
	Pen a 1 44^{R} 53^{I}	.R........T..	Pen a 1 45^{A} 51^{K}	..A.....K....
	Pen a 1 49^{K} 53^{I}	......K...I..	Pen a 1 45^{A} 52^{G}	..A......G...
	Pen a 1 44^{R} 45^{A}	.RA..........	Pen a 1 45^{A} 53^{I}	..A.......I..
	Pen a 1 44^{R} 49^{K}	.R....K......	Pen a 1 46^{M} 53^{T}	...M......T..
	Pen a 1 44^{R} 50^{I}	.R.....I.....	Pen a 1 49^{N} 50^{L}	......NL.....
	Pen a 1 44^{R} 53^{I}	.R........I..	Pen a 1 49^{K} 50^{L}	......KL.....
	Pen a 1 49^{K} 53^{I}	......K...I..	Pen a 1 49^{K} 50^{I}	......KI.....
	Pen a 1 43^{L} 44^{V}	LV...........	Pen a 1 49^{K} 52^{A}	......K..A...
	Pen a 1 43^{L} 49^{K}	L.....K......	Pen a 1 49^{K} 55^{D}	......K.....D
	Pen a 1 43^{L} 51^{K}	L.......K....	Pen a 1 50^{L} 51^{K}	.......LK....
	Pen a 1 43^{L} 52^{G}	L........G...	Pen a 1 50^{L} 52^{G}	.......L.G...
	Pen a 1 43^{L} 53^{T}	L.........T..	Pen a 1 50^{I} 53^{I}	.......I..I..
	Pen a 1 44^{V} 45^{A}	.VA..........	Pen a 1 51^{K} 55^{D}	........K...D
	Pen a 1 44^{V} 49^{K}	.V....K......	Pen a 1 52^{G} 55^{D}	.........G..D
	Pen a 1 44^{V} 51^{K}	.V......K....	Pen a 1 52^{A} 55^{D}	.........A..D
	Pen a 1 44^{V} 52^{G}	.V.......G...		
	Pen a 1 44^{V} 53^{T}	.V........T..		

Table 5 (a)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 1 (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 43-55	VHNLQKRMQQLEN	Pen a 1 43-55	VHNLQKRMQQLEN
mutated sequences containing 2 substitutions	Pen a 1 44^{V} 55^{D}	.V..........D		
	Pen a 1 45^{A} 53^{T}	..A.......T..		
	Pen a 1 49^{K} 51^{K}	......K.K....		
	Pen a 1 49^{K} 52^{G}	......K..G...		
	Pen a 1 49^{K} 53^{T}	......K...T..		
	Pen a 1 50^{L} 53^{T}	.......L..T..		
	Pen a 1 51^{K} 52^{G}	........KG...		
	Pen a 1 51^{K} 53^{T}	........K.T..		
	Pen a 1 52^{G} 53^{T}	.........GT..		
	Pen a 1 53^{T} 55^{D}	..........T.D		
	Pen a 1 43^{Q} 44^{Q}	QQ...........		
	Pen a 1 43^{Q} 45^{G}	Q.G..........		
	Pen a 1 43^{Q} 49^{K}	Q.....K......		
	Pen a 1 43^{Q} 51^{K}	Q.......K....		
	Pen a 1 43^{Q} 52^{G}	Q........G...		
	Pen a 1 43^{Q} 53^{T}	Q.........T..		
	Pen a 1 43^{Q} 55^{D}	Q...........D		
	Pen a 1 44^{Q} 45^{G}	.QG..........		
	Pen a 1 44^{Q} 49^{K}	.Q....K......		
	Pen a 1 44^{Q} 51^{K}	.Q......K....		
	Pen a 1 44^{Q} 52^{G}	.Q.......G...		
	Pen a 1 44^{Q} 53^{T}	.Q........T..		
	Pen a 1 44^{Q} 55^{D}	.Q..........D		
	Pen a 1 45^{G} 51^{K}	..G.....K....		
	Pen a 1 45^{G} 52^{G}	..G......G...		
	Pen a 1 45^{G} 53^{T}	..G.......T..		
	Pen a 1 44^{V} 45^{S}	.VS..........		
	Pen a 1 44^{V} 52^{A}	.V.......A...		

Table 5 (a)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 1 (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 43-55	VHNLQKRMQQLEN	Pen a 1 43-55	VHNLQKRMQQLEN
mutated sequences containing 2 substitutions	Pen a 1 45^{S} 49^{K}	..S...K......		
	Pen a 1 45^{S} 51^{K}	..S.....K....		
	Pen a 1 45^{S} 53^{T}	..S.......T..		
	Pen a 1 51^{K} 52^{A}	........KA...		
	Pen a 1 52^{A} 53^{T}	.........AT..		
	Pen a 1 44^{Q} 45^{A}	.QA..........		
	Pen a 1 43^{L} 44^{I}	LI...........		
	Pen a 1 43^{L} 46^{M}	L..M.........		
	Pen a 1 44^{I} 45^{Q}	.IQ..........		
	Pen a 1 44^{I} 46^{M}	.I.M.........		
	Pen a 1 44^{I} 49^{K}	.I....K......		
	Pen a 1 44^{I} 51^{K}	.I......K....		
	Pen a 1 44^{I} 52^{G}	.I.......G...		
	Pen a 1 44^{I} 53^{T}	.I........T..		
	Pen a 1 44^{I} 55^{D}	.I..........D		
	Pen a 1 45^{Q} 46^{M}	..QM.........		
	Pen a 1 45^{Q} 53^{T}	..Q.......T..		
	Pen a 1 46^{M} 49^{K}	...M..K......		
	Pen a 1 46^{M} 50^{L}	...M...L.....		
	Pen a 1 46^{M} 51^{K}	...M....K....		
	Pen a 1 46^{M} 52^{G}	...M.....G...		
	Pen a 1 46^{M} 55^{D}	...M........D		
	Pen a 1 43^{L} 44^{L}	LL...........		
	Pen a 1 43^{L} 49^{N}	L.....N......		
	Pen a 1 44^{L} 45^{S}	.LS..........		
	Pen a 1 44^{L} 49^{N}	.L....N......		
	Pen a 1 44^{L} 51^{K}	.L......K....		
	Pen a 1 44^{L} 52^{G}	.L.......G...		

Table 5 (a)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 1 (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 43-55	VHNLQKRMQQLEN	Pen a 1 43-55	VHNLQKRMQQLEN
mutated sequences containing 2 substitutions	Pen a 1 44^{L} 53^{T}	.L........T..		
	Pen a 1 44^{L} 55^{D}	.L..........D		
	Pen a 1 45^{S} 49^{N}	..S...N......		
	Pen a 1 49^{N} 51^{K}	......N.K....		
	Pen a 1 49^{N} 52^{G}	......N..G...		
	Pen a 1 49^{N} 53^{T}	......N...T..		
	Pen a 1 49^{N} 55^{D}	......N.....D		

Table 5 (b)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 2			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolishedIgE reactivity	
	sequence name	sequence	sequence name	sequence
unmodified Pen a 1 sequence	Pen a 1 87-101	ALNRRIQLLEEDLER	Pen a 1 87-101	ALNRRIQLLEEDLER
mutated sequences containing 1 substitution	Pen a 1 95^{V}	........V......	Pen a 1 87^{G}	G..............
			Pen a 1 95^{I}	........I......
			Pen a 1 95^{F}	........F......
			Pen a 1 98^{E}	...........E...
			Pen a 1 100^{D}	.............D.
mutated sequences containing 2 substitutions	Pen a 1 87^{S} 95^{V}	S.......V......	Pen a 1 87^{S} 98^{E}	S..........E...
	Pen a 1 95^{V} 98^{E}	........V..E...	Pen a 1 87^{S} 100^{D}	S............D.
	Pen a 1 95^{V} 100^{D}	........V....D.	Pen a 1 95^{F} 100^{D}	........F....D.
	Pen a 1 98^{E} 100^{D}	...........E.D.		
	Pen a 1 87^{S} 95^{F}	S.......F......		
	Pen a 1 95^{F} 98^{E}	........F..E...		
	Pen a 1 87^{G} 95^{V}	G.......V......		
	Pen a 1 87^{G} 98^{E}	G..........E...		
	Pen a 1 87^{G} 100^{D}	G............D.		
mutated sequences containing 3 substitutions	Pen a 1 87^{G} 95^{V} 98^{E}	G.......V..E...		
	Pen a 1 87^{G} 95^{V} 100^{D}	G.......V....D.		
	Pen a 1 87^{S} 95^{V} 98^{E}	S.......V..E...		
	Pen a 1 87^{S} 98^{E} 100^{D}	S..........E.D.		
	Pen a 1 95^{V} 98^{E} 100^{D}	........V..E.D.		
	Pen a 1 87^{S} 95^{F} 98^{E}	S.......F..E...		
	Pen a 1 87^{S} 95^{F} 100^{D}	S.......F....D.		
	Pen a 1 95^{F} 98^{E} 100^{D}	........F..E.D.		
	Pen a 1 87^{G} 95^{V} 98^{E}	G.......V..E...		
	Pen a 1 87^{G} 95^{V} 100^{D}	G.......V....D.		
	Pen a 1 87^{G} 98^{E} 100^{D}	G..........E.D.		
mutated sequences containing 4 substitutions	Pen a 1 87^{S} 95^{V} 98^{E} 100^{D}	S.......V..E.D.		
	Pen a 1 87^{S} 95^{F} 98^{E} 100^{D}	S.......F..E.D.		

Table 5(5)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 3a			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolishedIgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 133-147	RSLSDEERMDALEN	Pen a 1 133-147	RSLSDEERMDALEN
mutated sequences containing 1 substitution			Pen a 1 136^{T}	...T..........
			Pen a 1 136^{K}	...K..........
			Pen a 1 136^{A}	...A..........
			Pen a 1 140^{K}	.......K......
			Pen a 1 142^{E}	.........E....
			Pen a 1 143^{I}	..........I...
			Pen a 1 143^{G}	..........G...
			Pen a 1 145^{D}	............D.
			Pen a 1 146^{I}	I
mutated sequences containing 2 substitutions	Pen a 1 133^{K} 134^{G}	KG............	Pen a 1 133^{K} 136^{A}	K..A..........
	Pen a 1 134^{G} 136^{A}	.G.A..........	Pen a 1 134^{A} 140^{K}	.A.....K......
	Pen a 1 134^{A} 135^{S}	.AS...........	Pen a 1 135^{S} 143^{L}	..S.......L...
	Pen a 1 134^{A} 135^{Q}	.AQ...........	Pen a 1 135^{Q} 143^{I}	..Q.......I...
	Pen a 1 134^{A} 135^{M}	.AM...........	Pen a 1 135^{Q} 146^{I}	..Q..........I
	Pen a 1 134^{A} 136^{K}	.A.K..........	Pen a 1 135^{M} 140^{K}	..M....K......
	Pen a 1 135^{S} 136^{K}	..SK..........	Pen a 1 135^{I} 136^{T}	..IT..........
	Pen a 1 135^{S} 140^{K}	..S....K......	Pen a 1 136^{K} 143^{L}	...K......L...
	Pen a 1 135^{S} 142^{E}	..S......E....	Pen a 1 140^{K} 143^{L}	.......K..L...
	Pen a 1 135^{S} 144^{Q}	..S........Q..	Pen a 1 140^{K} 143^{I}	.......K..I...
	Pen a 1 135^{S} 145^{D}	..S.........D.	Pen a 1 140^{K} 146^{M}	.......K.....M
	Pen a 1 135^{S} 146^{I}	..S..........I	Pen a 1 140^{K} 146^{I}	.......K.....I
	Pen a 1 135^{Q} 136^{K}	..QK..........	Pen a 1 142^{E} 145^{D}	.........E..D.
	Pen a 1 135^{Q} 140^{K}	..Q....K......	Pen a 1 143^{L} 145^{D}	..........L.D.
	Pen a 1 135^{Q} 142^{E}	..Q......E....	Pen a 1 143^{L} 146^{M}	..........L..M
	Pen a 1 135^{Q} 144^{Q}	..Q........Q..	Pen a 1 143^{L} 146^{I}	..........L..I
	Pen a 1 135^{M} 136^{K}	..MK..........	Pen a 1 143^{I} 146^{I}	..........I..I

Table 5 (c)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 3a (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 133-147	RSLSDEERMDALEN	Pen a 1 133-147	RSLSDEERMDALEN
mutated sequences containing 2 substitutions	Pen a 1 135^{M} 142^{E}	..M......E....	Pen a 1 144^{Q} 145^{D}	...........QD.
	Pen a 1 135^{M} 144^{Q}	..M........Q..	Pen a 1 145^{D} 146^{I}	............DI
	Pen a 1 135^{I} 142^{E}	..I......E....		
	Pen a 1 135^{I} 143^{G}	..I.......G...		
	Pen a 1 136^{T} 142^{E}	...T.....E....		
	Pen a 1 136^{T} 143^{G}	...T......G...		
	Pen a 1 136^{K} 140^{K}	...K...K......		
	Pen a 1 136^{K} 142^{E}	...K.....E....		
	Pen a 1 136^{K} 143^{I}	...K......I...		
	Pen a 1 136^{K} 144^{Q}	...K.......Q..		
	Pen a 1 136^{K} 145^{D}	...K........D.		
	Pen a 1 136^{K} 146^{M}	...K.........M		
	Pen a 1 136^{K} 146^{I}	...K.........I		
	Pen a 1 140^{K} 142^{E}	.......K.E....		
	Pen a 1 140^{K} 144^{Q}	.......K...Q..		
	Pen a 1 140^{K} 145^{D}	.......K....D.		
	Pen a 1 142^{E} 143^{L}	.........EL...		
	Pen a 1 142^{E} 143^{I}	.........EI...		
	Pen a 1 142^{E} 143^{G}	.........EG...		
	Pen a 1 142^{E} 144^{Q}	.........E.Q..		
	Pen a 1 142^{E} 146^{M}	.........E...M		
	Pen a 1 142^{E} 146^{I}	.........E...I		
	Pen a 1 143^{L} 144^{Q}	..........LQ..		
	Pen a 1 143^{I} 144^{Q}	..........IQ..		
	Pen a 1 144^{Q} 146^{M}	...........Q.M		
	Pen a 1 144^{Q} 146^{I}	...........Q.I		

Table 5 (c)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 3a (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 133-147	RSLSDEERMDALEN	Pen a 1 133-147	RSLSDEERMDALEN
mutated sequences containing 3 substitutions	Pen a 1 133^{K} 134^{G} 136-A	KG.A..........		
	Pen a 1 134^{A} 135^{S} 136-K	.ASK..........		
	Pen a 1 134^{A} 135^{S} 140-K	.AS....K......		
	Pen a 1 134^{A} 135^{Q} 136-K 140-K*	.AQK...K......		
	Pen a 1 134^{A} 135^{Q} 136-K	.AQK..........		
	Pen a 1 134^{A} 135^{Q} 140-K	.AQ....K......		
	Pen a 1 134^{A} 135^{M} 136-K	.AMK..........		
	Pen a 1 134^{A} 135^{M} 140-K	.AM....K......		
	Pen a 1 134^{A} 136^{K} 140-K	.A.K...K......		
	Pen a 1 135^{S} 136^{K} 140-K	..SK...K......		
	Pen a 1 135^{Q} 136^{K} 140-K	..QK...K......		
	Pen a 1 135^{M} 136^{K} 140-K	..MK...K......		
	Pen a 1 135^{I} 136^{T} 142-E	..IT.....E....		
	Pen a 1 135^{I} 136^{T} 143-G	..IT......G...		
	Pen a 1 135^{I} 142^{E} 143-G	..I......EG...		
	Pen a 1 136^{T} 142^{E} 143-G	...T.....EG...		
	Pen a 1 136^{K} 140^{K} 142-E	...K...K.E....		
	Pen a 1 136^{K} 140^{K} 143-L	...K...K..L...		
	Pen a 1 136^{K} 140^{K} 143-I	...K...K..I...		
	Pen a 1 136^{K} 142^{E} 143-L	...K.....EL...		
	Pen a 1 136^{K} 142^{E} 143-I	...K.....EI...		
	Pen a 1 140^{K} 142^{E} 143-L	.......K.EL...		
	Pen a 1 140^{K} 142^{E} 143-I	.......K.EI...		
	Pen a 1 140^{K} 142^{E} 144-Q	.......K.E.Q..		
	Pen a 1 140^{K} 142^{E} 145-D	.......K.E..D.		
	Pen a 1 140^{K} 143^{L} 144-Q	.......K..LQ..		

Table 5(c)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 3a (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 133-147	RSLSDEERMDALEN	Pen a 1 133-147	RSLSDEERMDALEN
mutated sequences containing 3 substitutions	Pen a 1 140^K 143^L 145-D	.......K..L.D.		
	Pen a 1 140^K 143^I 144-Q	.......K..IQ..		
	Pen a 1 140^K 144^Q 145-D	.......K...QD.		
	Pen a 1 142^E 143^L 144-Q	.........ELQ..		
	Pen a 1 142^E 143^L 145-D	.........EL.D.		
	Pen a 1 142^E 143^I 144-Q	.........EIQ..		
	Pen a 1 142^E 144^Q 145-D	.........E.QD.		
	Pen a 1 143^L 144^Q 145-D	..........LQD.		
mutated sequences containing 4 substitutions	Pen a 1 134^A 135^S 136-K 140-K*	.ASK...K......		
	Pen a 1 134^A 135^Q 136-K 140-K*	.AQK...K......		
	Pen a 1 134^A 135^M 136-K 140-K*	.AMK...K......		
	Pen a 1 135^I 136^T 142-E 143-G*	..IT.....EG...		
	Pen a 1 136^K 140^K 142-E 143-I*	...K...K.EI...		
	Pen a 1 140^K 142^E 143-L 145-D*	.......K.EL.D.		
	Pen a 1 140^K 142^E 143-I 144-Q*	.......K.EIQ..		
	Pen a 1 140^K 142^E 144-Q 145-D*	.......K.E.QD.		
	Pen a 1 140^K 143^L 144-Q 145-D*	.......K..LQD.		
	Pen a 1 142^E 143^L 144-Q 145-D*	.........ELQD.		

Table 5(d)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 3b			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	peptide name	peptide sequence	peptide name	peptide sequence
unmodified Pen a 1 sequence	Pen a 1 139-153	ERMDALENQLKEARF	Pen a 1 139-153	ERMDALENQLKEARF
mutated sequences containing 1 substitution	Pen a 1 143^{G}	....G..........		
	Pen a 1 144^{Q}	.....Q.........		
	Pen a 1 145^{D}	......D........		
	Pen a 1 153^{M}	..............M		
	Pen a 1 153^{H}	..............H		
mutated sequences containing 2 substitutions	Pen a 1 140^{K} 144^{Q}	.K...Q.........	Pen a 1 140^{K} 153^{H}	.K............H
	Pen a 1 140^{K} 145^{D}	.K....D........	Pen a 1 142^{E}	...E...........
	Pen a 1 142^{E} 143^{G}	...EG..........	Pen a 1 142^{E} 144^{Q}	...E.Q.........
	Pen a 1 142^{E} 145^{D}	...E..D........	Pen a 1 142^{E} 153^{H}	...E..........H
	Pen a 1 142^{E} 146^{M}	...E...M.......	Pen a 1 143^{L} 144^{Q}	....LQ.........
	Pen a 1 142^{E} 146^{I}	...E...I.......	Pen a 1 143^{L} 145^{D}	....L.D........
	Pen a 1 142^{E} 153^{M}	...E..........M	Pen a 1 143^{L} 146^{I}	....L..I.......
	Pen a 1 143^{L} 146^{M}	....L..M.......	Pen a 1 143^{L} 153^{H}	....L.........H
	Pen a 1 143^{I} 146^{I}	....I..I.......	Pen a 1 143^{I} 144^{Q}	....IQ.........
	Pen a 1 143^{I} 153^{H}	....I.........H	Pen a 1 143^{I} 152^{K}	....I........K.
	Pen a 1 143^{G} 153^{M}	....G.........M	Pen a 1 146^{M} 152^{K}	.......M.....K.
	Pen a 1 144^{Q} 145^{D}	.....QD........	Pen a 1 146^{M}	.......M.......
	Pen a 1 144^{Q} 146^{M}	.....Q.M.......	Pen a 1 146^{I} 152^{K}	.......I.....K.
	Pen a 1 144^{Q} 146^{I}	.....Q.I.......	Pen a 1 146^{I} 153^{H}	.......I......H
	Pen a 1 144^{Q} 152^{K}	.....Q.......K.		
	Pen a 1 144^{Q} 153^{H}	.....Q........H		
	Pen a 1 145^{D} 146^{I}	......DI.......		
	Pen a 1 145^{D} 152^{K}	......D......K.		
	Pen a 1 145^{D} 153^{H}	......D.......H		
	Pen a 1 146^{M} 153^{H}	.......M......H		
	Pen a 1 152^{K} 153^{H}	.............KH		

Table 5(e)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	penal 249-261	LQKEVDRLEDELV	penal 249-261	LQKEVDRLEDELV
mutated sequences containing 1 substitution	penal 255^{D}	......D......	penal 250^{E}	.E...........
	penal 260^{V}	...........V.	penal 252^{T}	...T.........
	penal 261^{Y}	............Y	penal 252^{S}	...S.........
			penal 253^{I}	....I........
mutated sequences containing 2 substitutions	penal 250^{E} 252^{S}	.E.S.........	penal 250^{E} 252^{T}	.E.T.........
	penal 250^{E} 255^{D}	.E....D......	penal 250^{E} 253^{I}	.E..I........
	penal 250^{E} 260^{V}	.E.........V.	penal 250^{E} 255^{D}	.E....D......
	penal 252^{T} 255^{D}	...T..D......	penal 252^{T} 253^{I}	...TI........
	penal 252^{T} 260^{V}	...T.......V.	penal 252^{S} 253^{I}	...SI........
	penal 252^{S} 255^{D}	...S..D......	penal 253^{I} 261^{Y}	....I.......Y
	penal 252^{S} 261^{Y}	...S........Y		
	penal 253^{I} 255^{D}	....I.D......		
	penal 253^{I} 260^{V}	....I......V.		
	penal 255^{D} 260^{V}	......D....V.		
	penal 255^{D} 261^{Y}	......D.....Y		
	penal 260^{V} 261^{Y}	...........VY		
mutated sequences containing 3 substitutions	penal 250^{E} 252^{T} 253^{I}	.E.TI........		
	penal 250^{E} 252^{T} 255^{D}	.E.T..D......		
	penal 250^{E} 252^{T} 260^{V}	.E.T.......V.		
	penal 250^{E} 252^{T} 261^{Y}	.E.T........Y		
	penal 250^{E} 252^{S} 253^{I}	.E.SI........		
	penal 250^{E} 252^{S} 255^{D}	.E.S..D......		
	penal 250^{E} 252^{S} 261^{Y}	.E.S........Y		
	penal 250^{E} 253^{I} 255^{D}	.E..I.D......		
	penal 250^{E} 253^{I} 260^{V}	.E..I......V.		
	penal 250^{E} 253^{I} 261^{Y}	.E..I.......Y		
	penal 250^{E} 255^{D} 260^{V}	.E....D....V.		

Table (5e)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	penal 249-261	LQKEVDRLEDELV	penal 249-261	LQKEVDRLEDELV
mutated sequences containing 3 substitutions	penal 250^{E} 255^{D} 261^{Y}	.E....D.....Y		
	penal 250^{E} 260^{V} 261^{Y}	.E.........VY		
	penal 252^{T} 253^{I} 255^{D}	...TI.D......		
	penal 252^{T} 253^{I} 260^{V}	...TI......V.		
	penal 252^{T} 255^{D} 260^{V}	...T..D....V.		
	penal 252^{T} 255^{D} 261^{Y}	...T..D.....Y		
	penal 252^{T} 260^{V} 261^{Y}	...T.......VY		
	penal 252^{S} 253^{I} 255^{D}	...SI.D......		
	penal 252^{S} 253^{I} 261^{Y}	...SI.......Y		
	penal 252^{S} 255^{D} 261^{Y}	...S..D.....Y		
	penal 253^{I} 255^{D} 260^{V}	....I.D....V.		
	penal 253^{I} 255^{D} 261^{Y}	....I.D.....Y		
	penal 253^{I} 260^{V} 261^{Y}	....I......VY		
	penal 255^{D} 260^{V} 261^{Y}	......D....VY		
mutated sequences containing 4 substitutions	penal 250^{E} 252^{T} 253^{I} 255^{D}	.E.TI.D......		
	penal 250^{E} 252^{T} 253^{I} 260^{V}	.E.TI......V.		
	penal 250^{E} 252^{T} 253^{I} 261^{Y}	.E.TI.......Y		
	penal 250^{E} 252^{T} 255^{D} 260^{V}	.E.T..D....V.		
	penal 250^{E} 252^{T} 255^{D} 261^{Y}	.E.T..D.....Y		
	penal 250^{E} 252^{T} 260^{V} 261^{Y}	.E.T.......VY		
	penal 250^{E} 252^{S} 253^{I} 255^{D}	.E.SI.D......		
	penal 250^{E} 252^{S} 253^{I} 261^{Y}	.E.SI.......Y		
	penal 250^{E} 252^{S} 255^{D} 261^{Y}	.E.S..D.....Y		
	penal 250^{E} 253^{I} 255^{D} 260^{V}	.E..I.D....V.		
	penal 250^{E} 253^{I} 260^{V} 261^{Y}	.E..I......VY		

Table (5e)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	pena1 249-261	LQKEVDRLEDELV	pena1 249-261	LQKEVDRLEDELV
mutated sequences containing 4 substitutions	pena1 250^{E} 255^{D} 260^{V} 261^{Y}	.E....D....VY		
	pena1 252^{T} 253^{I} 255^{D} 260^{V}	...TI.D....V.		
	pena1 252^{T} 253^{I} 255^{D} 261^{Y}	...TI.D.....Y		
	pena1 252^{T} 253^{I} 260^{V} 261^{Y}	...TI......VY		
	pena1 252^{T} 255^{D} 260^{V} 261^{Y}	...T..D....VY		
	pena1 253^{I} 255^{D} 260^{V} 261^{Y}	....I.D....VY		
mutated sequences containing 5 substitutions	pena1 250^{E} 252^{T} 253^{I} 255^{D} 260^{V}	.E.TI.D....V.		
	pena1 250^{E} 252^{T} 253^{I} 255^{D} 261^{Y}	.E.TI.D.....Y		
	pena1 250^{E} 252^{T} 253^{I} 260^{V} 261^{Y}	.E.TI......VY		
	pena1 250^{E} 252^{T} 255^{D} 260^{V} 261^{Y}	.E.T..D....VY		
	pena1 250^{E} 253^{I} 255^{D} 260^{V} 261^{Y}	.E..I.D....VY		
	pena1 252^{T} 253^{I} 255^{D} 260^{V} 261^{Y}	...TI.D....VY		

Table 5(e)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 249-261	LQKEVDRLEDELV	Pen a 1 249-261	LQKEVDRLEDELV
mutated sequences containing 1 substitution	Pen a 1 255^{D}	......D......	Pen a 1 250^{E}	.E...........
	Pen a 1 260^{V}	...........V.	Pen a 1 252^{T}	...T.........
	Pen a 1 261^{Y}	............Y	Pen a 1 252^{S}	...S.........
			Pen a 1 253^{I}	....I........
mutated sequences containing 2 substitutions	Pen a 1 250^{E} 252^{S}	.E.S.........	Pen a 1 250^{E} 252^{T}	.E.T.........
	Pen a 1 250^{E} 255^{D}	.E....D......	Pen a 1 250^{E} 253^{I}	.E..I........
	Pen a 1 250^{E} 260^{V}	.E.........V.	Pen a 1 250^{E} 255^{D}	.E....D......
	Pen a 1 252^{T} 255^{D}	...T..D......	Pen a 1 252^{T} 253^{I}	...TI........
	Pen a 1 252^{T} 260^{V}	...T.......V.	Pen a 1 252^{S} 253^{I}	...SI........
	Pen a 1 252^{S} 255^{D}	...S..D......	Pen a 1 253^{I} 261^{Y}	....I.......Y
	Pen a 1 252^{S} 261^{Y}	...S........Y		
	Pen a 1 253^{I} 255^{D}	....I.D......		
	Pen a 1 253^{I} 260^{V}	....I......V.		
	Pen a 1 255^{D} 260^{V}	......D....V.		
	Pen a 1 255^{D} 261^{Y}	......D.....Y		
	Pen a 1 260^{V} 261^{Y}	...........VY		
mutated sequences containing 3 substitutions	Pen a 1 250^{E} 252^{T} 253^{I}	.E.TI........		
	Pen a 1 250^{E} 252^{T} 255^{D}	.E.T..D......		
	Pen a 1 250^{E} 252^{T} 260^{V}	.E.T.......V.		
	Pen a 1 250^{E} 252^{T} 261^{Y}	.E.T........Y		
	Pen a 1 250^{E} 252^{S} 253^{I}	.E.SI........		
	Pen a 1 250^{E} 252^{S} 255^{D}	.E.S..D......		
	Pen a 1 250^{E} 252^{S} 261^{Y}	.E.S........Y		
	Pen a 1 250^{E} 253^{I} 255^{D}	.E..I.D......		
	Pen a 1 250^{E} 253^{I} 260^{V}	.E..I......V.		
	Pen a 1 250^{E} 253^{I} 261^{Y}	.E.[illegible]......Y		
	Pen a 1 250^{E} 255^{D} 260^{V}	.E.[illegible]D....V.		

Table 5(e)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolishedIgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 249-261	LQKEVDRLEDELV	Pen a 1 249-261	LQKEVDRLEDELV
mutated sequences containing 3 substitutions	Pen a 1 250^{E} 255^{D} 261^{Y}	.E....D.....Y		
	Pen a 1 250^{E} 260^{V} 261^{Y}	.E.........VY		
	Pen a 1 252^{T} 253^{I} 255^{D}	...TI.D......		
	Pen a 1 252^{T} 253^{I} 260^{V}	...TI......V.		
	Pen a 1 252^{T} 255^{D} 260^{V}	...T..D....V.		
	Pen a 1 252^{T} 255^{D} 261^{Y}	...T..D.....Y		
	Pen a 1 252^{T} 260^{V} 261^{Y}	...T.......VY		
	Pen a 1 252^{S} 253^{I} 255^{D}	...SI.D......		
	Pen a 1 252^{S} 253^{I} 261^{Y}	...SI.......Y		
	Pen a 1 252^{S} 255^{D} 261^{Y}	...S..D.....Y		
	Pen a 1 253^{I} 255^{D} 260^{V}	....I.D....V.		
	Pen a 1 253^{I} 255^{D} 261^{Y}	....I.D.....Y		
	Pen a 1 253^{I} 260^{V} 261^{Y}	....I......VY		
	Pen a 1 255^{D} 260^{V} 261^{Y}	......D....VY		
mutated sequences containing 4 substitutions	Pen a 1 250^{E} 252^{T} 253^{I} 255^{D}	.E.TI.D......		
	Pen a 1 250^{E} 252^{T} 253^{I} 260^{V}	.E.TI......V.		
	Pen a 1 250^{E} 252^{T} 253^{I} 261^{Y}	.E.TI.......Y		
	Pen a 1 250^{E} 252^{T} 255^{D} 260^{V}	.E.T..D....V.		
	Pen a 1 250^{E} 252^{T} 255^{D} 261^{Y}	.E.T..D.....Y		
	Pen a 1 250^{E} 252^{T} 260^{V} 261^{Y}	.E.T.......VY		
	Pen a 1 250^{E} 252^{S} 253^{I} 255^{D}	.E.SI.D......		
	Pen a 1 250^{E} 252^{S} 253^{I} 261^{Y}	.E.SI.......Y		
	Pen a 1 250^{E} 252^{S} 255^{D} 261^{Y}	.E.S..D.....Y		
	Pen a 1 250^{E} 253^{I} 255^{D} 260^{V}	.E..I.D....V.		
	Pen a 1 250^{E} 253^{I} 260^{V} 261^{Y}	.E..I......VY		

Table 5(e)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 249-261	LQKEVDRLEDELV	Pen a 1 249-261	LQKEVDRLEDELV
mutated sequences containing 4 substitutions	Pen a 1 250^{E} 255^{D} 260^{V} 261^{Y}	.E....D....VY		
	Pen a 1 252^{T} 253^{I} 255^{D} 260^{V}	...TI.D....V.		
	Pen a 1 252^{T} 253^{I} 255^{D} 261^{Y}	...TI.D.....Y		
	Pen a 1 252^{T} 253^{I} 260^{V} 261^{Y}	...TI......VY		
	Pen a 1 252^{T} 255^{D} 260^{V} 261^{Y}	...T..D....VY		
	Pen a 1 253^{I} 255^{D} 260^{V} 261^{Y}	....I.D....VY		
mutated sequences containing 5 substitutions	Pen a 1 250^{E} 252^{T} 253^{I} 255^{D} 260^{V}	.E.TI.D....V.		
	Pen a 1 250^{E} 252^{T} 253^{I} 255^{D} 261^{Y}	.E.TI.D.....Y		
	Pen a 1 250^{E} 252^{T} 253^{I} 260^{V} 261^{Y}	.E.TI......VY		
	Pen a 1 250^{E} 252^{T} 255^{D} 260^{V} 261^{Y}	.E.T..D....VY		
	Pen a 1 250^{E} 253^{I} 255^{D} 260^{V} 261^{Y}	.E..I.D....VY		
	Pen a 1 252^{T} 253^{I} 255^{D} 260^{V} 261^{Y}	...TI.D....VY		

Table 5(e)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
unmodified Pen a 1 sequence	Pen a 1 273-281	ELDQTFSEL	Pen a 1 273-281	ELDQTFSEL
mutated sequences containing substitution	Pen a 1 277^{A}	....A....	Pen a 1 276^{M}	...M.....
	Pen a 1 278^{L}	.....L...	Pen a 1 275^{M} 281	..M.....
	Pen a 1 280^{D}	.......D.		
	Pen a 1 281^{M}	........M		
	Pen a 1 281^{I}	I		
mutated sequences containing 2 substitutions	Pen a 1 276^{H} 277^{A}	...HA....	Pen a 1 276^{M} 279^{T}	...M..T..
	Pen a 1 276^{H} 278^{L}	...H.L...	Pen a 1 276^{H} 279^{N}	...H..N..
	Pen a 1 276^{H} 280^{D}	...H...D.	Pen a 1 276^{H} 279^{N}	...H..N..
	Pen a 1 276^{H} 281^{M}	...H....M		
	Pen a 1 277^{A} 278^{L}	....AL...		
	Pen a 1 277^{A} 279^{N}	....A.N..		
	Pen a 1 277^{A} 280^{D}	....A..D.		
	Pen a 1 277^{A} 281^{M}	....A...M		
	Pen a 1 278^{L} 279^{N}	.....LN..		

Table 5(b)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5b (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 266-273	KYKSITDE	Pen a 1 266-273	KYKSITDE
mutated sequences containing 1 substitution	Pen a 1 269^{E}	...E....		
	Pen a 1 269^{D}	...D....		
	Pen a 1 269^{C}	...C....		
	Pen a 1 269^{A}	...A....		
	Pen a 1 270^{W}	....W...		
	Pen a 1 270^{T}	....T...		
	Pen a 1 270^{S}	....S...		
	Pen a 1 270^{R}	....R...		
	Pen a 1 270^{Q}	....Q...		
	Pen a 1 270^{P}	....P...		
	Pen a 1 270^{N}	....N...		
	Pen a 1 270^{M}	....M...		
	Pen a 1 270^{K}	....K...		
	Pen a 1 270^{H}	....H...		
	Pen a 1 270^{G}	....G...		
	Pen a 1 270^{F}	....F...		
	Pen a 1 270^{E}	....E...		
	Pen a 1 270^{D}	....D...		
	Pen a 1 270^{C}	....C...		
	Pen a 1 270^{A}	....A...		
	Pen a 1 271^{P}	.....P..		
	Pen a 1 272^{R}	......R.		
	Pen a 1 272^{P}	......P.		
	Pen a 1 272^{K}	......K.		

Table (5f)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5b (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolishedIgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 266-273	KYKSITDE	Pen a 1 266-273	KYKSITDE
mutated sequences containing 2 substitutions	Pen a 1 269^F 271^C	...F.C..	Pen a 1 269^A 271^S	...A.S..
	Pen a 1 269^F 273^D	...F...D	Pen a 1 269^A 272^E	...A..E.
			Pen a 1 271^S 272^E	.....SE.
			Pen a 1 271^C 273^D	.....C.D
mutated sequences containing 3 substitutions	Pen a 1 269^F 271^C 273^D	...F.C.D	Pen a 1 269^A 271^S 272^E	...A.SE.

Table 5(f)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5b			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 266-273	KYKSITDE	Pen a 1 266-273	KYKSITDE
mutated sequences containing 1 substitution	Pen a 1 269^{F}	...F....	Pen a 1 269^{A}	...A....
			Pen a 1 271^{S}	.....S..
	Pen a 1 266^{Y}	Y.......	Pen a 1 266^{N}	N.......
	Pen a 1 266^{W}	W.......	Pen a 1 267^{H}	.H......
	Pen a 1 266^{V}	V.......	Pen a 1 268^{Y}	..Y.....
	Pen a 1 266^{T}	T.......	Pen a 1 268^{W}	..W.....
	Pen a 1 266^{S}	S.......	Pen a 1 268^{T}	..T.....
	Pen a 1 266^{R}	R.......	Pen a 1 268^{S}	..S.....
	Pen a 1 266^{Q}	Q.......	Pen a 1 268^{N}	..N.....
	Pen a 1 266^{P}	P.......	Pen a 1 268^{L}	..L.....
	Pen a 1 266^{M}	M.......	Pen a 1 268^{I}	..I.....
	Pen a 1 266^{L}	L.......	Pen a 1 268^{H}	..H.....
	Pen a 1 266^{I}	I.......	Pen a 1 268^{G}	..G.....
	Pen a 1 266^{H}	H.......	Pen a 1 268^{F}	..F.....
	Pen a 1 266^{G}	G.......	Pen a 1 268^{A}	..A.....
	Pen a 1 266^{F}	F.......	Pen a 1 269^{T}	...T....
	Pen a 1 266^{E}	E.......	Pen a 1 269^{G}	...G....
	Pen a 1 266^{D}	D.......	Pen a 1 270^{Y}	....Y...
	Pen a 1 266^{C}	C.......	Pen a 1 270^{V}	....V...
	Pen a 1 266^{A}	A.......	Pen a 1 270^{L}	....L...
	Pen a 1 267^{V}	.V......	Pen a 1 270^{F}	....F...
	Pen a 1 267^{T}	.T......	Pen a 1 270^{E}	....E...
	Pen a 1 267^{S}	.S......	Pen a 1 271^{W}	.....W..
	Pen a 1 267^{R}	.R......	Pen a 1 271^{K}	.....K..
	Pen a 1 267^{Q}	.Q......	Pen a 1 272^{Y}	......Y.
	Pen a 1 267^{P}	.P......	Pen a 1 272^{W}	......W.
	Pen a 1 267^{M}	.M......	Pen a 1 272^{V}	......V.

Table 5(f)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5b (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 266-273	KYKSITDE	Pen a 1 266-273	KYKSITDE
mutated sequences containing 1 substitution	Pen a 1 267^{L}	.L......	Pen a 1 272^{T}	......T.
	Pen a 1 267^{K}	.K......	Pen a 1 272^{M}	......M.
	Pen a 1 267^{I}	.I......	Pen a 1 272^{L}	......L.
	Pen a 1 267^{G}	.G......	Pen a 1 272^{I}	......I.
	Pen a 1 267^{E}	.E......	Pen a 1 272^{H}	......H.
	Pen a 1 267^{D}	.D......	Pen a 1 272^{F}	......F.
	Pen a 1 267^{C}	.C......	Pen a 1 272^{C}	......C.
	Pen a 1 267^{A}	.A......	Pen a 1 272^{A}	......A.
	Pen a 1 268^{V}	..V.....	Pen a 1 273^{Y}	.......Y
	Pen a 1 268^{P}	..P.....	Pen a 1 273^{W}	.......W
	Pen a 1 268^{E}	..E.....	Pen a 1 273^{V}	V
	Pen a 1 268^{D}	..D.....	Pen a 1 273^{T}	.......T
	Pen a 1 268^{C}	..C.....	Pen a 1 273^{S}	.......S
	Pen a 1 269^{Y}	...Y....	Pen a 1 273^{R}	.......R
	Pen a 1 269^{W}	...W....	Pen a 1 273^{Q}	.......Q
	Pen a 1 269^{V}	...V....	Pen a 1 273^{P}	.......P
	Pen a 1 269^{T}	...T....	Pen a 1 273^{N}	.......N
	Pen a 1 269^{R}	...R....	Pen a 1 273^{M}	.......M
	Pen a 1 269^{Q}	...Q....	Pen a 1 273^{L}	L
	Pen a 1 269^{P}	...P....	Pen a 1 273^{K}	.......K
	Pen a 1 269^{M}	...M....	Pen a 1 273^{I}	I
	Pen a 1 269^{L}	...L....	Pen a 1 273^{H}	.......H
	Pen a 1 269^{K}	...K....	Pen a 1 273^{G}	.......G
	Pen a 1 269^{I}	...I....	Pen a 1 273^{F}	.......F
	Pen a 1 269^{H}	...H....	Pen a 1 273^{A}	.......A
	Pen a 1 269^{G}	...G....		
	Pen a 1 269^{F}	...F....		

Table 5 (g)

	Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5c (continued)			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolishedIgE reactivity	
	sequence name	sequence	sequence name	sequence
unmodified Pen a 1 sequence	Pen a 1 273-281	ELDQTFSEL	Pen a 1 273-281	ELDQTFSEL
mutated sequences containing 2 substitutions	Pen a 1 280^{D} 281^{M}	.......DM		
	Pen a 1 276^{N} 277^{A}	...NA....		
	Pen a 1 276^{N} 278^{L}	...N.L...		
	Pen a 1 276^{N} 280^{D}	...N...D.		
	Pen a 1 276^{N} 281^{I}	...N....I		
	Pen a 1 277^{A} 281^{I}	....A...I		
	Pen a 1 278^{L} 281^{I}	.....L..I		
	Pen a 1 279^{N} 281^{I}	......N.I		
	Pen a 1 280^{D} 281^{I}	.......DI		
	Pen a 1 276^{N} 281^{M}	...N....M		
mutated sequences containing 3 substitutions			Pen a 1 273^{D} 276^{M} 279^{T}	D..M..T..

location	recombinant peptide library	overlapping, synthetic peptides
N-terminus		Pen a 1 1-15 MDAIKKKMQAMKLEK Pen a 1 79-93 SNAEGEVAALNRRIQ
center	Pen a 1 136-148 SDEERMDALENQL Pen a 1 157-169 EADRKYDEVARKL Pen a 1 167-179 RKLAMVEADLERA	Pen a 1 109-123 ATTKLAEASQAADES Pen a 1 121-135 DESERMRKVLENRSL Pen a 1 133-147 RSLSDEERMDALENQ Pen a 1 187-201 ESKIVELEEELRVVG
C-terminus	Pen a 1 262-282 NEKEKYKSITDELDQTFSELS	Pen a 1 217-231 REEAYKEQIKTLTNK Pen a 1 241-255 FAERSVQKLQKEVDR Pen a 1 253-267 VDRLEDELVNEKEKY

Table 6: IgE-reactive peptides of Pen a 1 identified with a recombinant peptide library and synthetic, overlapping peptides

Table 7

This table shows all the substitutions (position, pos; substituting amino acid, aa) that can be considered to reduce or abolish the IgE antibody reactivity of major and minor IgE-binding regions. In addition to the five major region, minor IgE-reactive regions, defined as regions to which at least one allergic subject shows strong IgE antibody reactivity.

minor region 1		major region 1		minor region 2		major region 2		minor region 3		minor region 4		major region 3		minor region 5		major region 4		minor region 6		major region 5	
1-33		43-57		61-75		87-101		103-117		121-135		133-153		145-188		187-207		220-230		249-284	
pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa
250	E	43	L	70	V	95	V	103	Q	125	G	135	K	146	I	188	G	220	K	250	E
252	S	44	I	71	V	95	F	107	A	127	M	140	K	153	K	190	C	222	E	252	S
255	D	45	Q	74	S	98	E	110	L	130	I	142	E	154	H	192	D	224	E	255	D
260	V	46	M			100	D	111	Q	134	A	144	Q	155	I	198	K	227	V	260	V
267	F	49	K					114	E	135	Q			163	E	199	T	230	D	267	F
277	A	50	L					117	E	135	Q			170	V	201	T			277	A
278	L	51	K							135	M			171	I					278	L
280	Q	52	G							135	S			172	I					280	Q
281	M	53	T											174	S					281	M
281	I	55	D											179	S					281	I
31	A	56	E											185	I						
33	E													186	S						
														188	G						

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30968

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.
US CL : 424/184.1, 536/23.5, 435/69.1, 435/320.1, 800/3, 530/387.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 536/23.5, 435/69.1, 435/320.1, 800/3, 530/387.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,449,669 A (METCALF ET AL) 12 September 1995(12/9/95), see entire document.	1-7, 12-26, 28-42
X	REESE, G. Int. Arch. Allergy Immunol. 1997, Vol 113, pages 240-242., See entire document.	8-11, 27, 42
X	REESE, G. J. Allergy Clin. Immunol. 1995, Vol 95, No. 1, Part 2, page 331. See abstract.	44
Y	US 6,118,044A (KARASUYAMA et al) 12 September 2000 (12/9/00), Col. 2, lines 14-39.	43

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 DECEMBER 2000	12 MAR 2001

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INTERNATIONAL SEARCH REPORT

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A61K 39/00, C07H 21/04, C12P 21/06, C12N 15/00, G01N 33/00, C07K 16/00

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN, MEDLINE, BIOSIS, CAPLUS, EMBASE, SCISEARCH
Vaccine, crustacea, tropomyosin, IgE, transgenic shrimp, crustacea tropomyosin antibody, recombinant peptide, allergen, IgE mediated hypersensitivity, Pen a 1